Societat Catalana de BIOLOGIA

# XXVII 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**

**26 de novembre de 2021**

Organitzadors:

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

Secretaria de la SCB:

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# PROGRAMA

**9:00 - 9:20** **Registration**

**9:20** **Welcoming and preliminary remarks**

**9:30** **Jens Lüders (IRB Barcelona)**.

*Microtubule nucleation complexes in unusual places*.

**10:15** **Short Talk 1. Laura Pastor (IBMB-CSIC)**.

*Crosstalk between CK2 & phosphoinositides***.**

**10:30** **Short Talk 2. Javier Jiménez (UIC)**.

*Polyphosphate degradation by Nudt3-$Zn^{2+}$ mediates oxidative stress response.*

**10:45** **Thomas Surrey (CRG)**.

*Lessons learnt from microtubules that cannot hydrolyze GTP.*

**11:30** **Short Break**

**11:45** **Short Talk 3. Gerard Cantero (VHIR)**.

*Reversing chemorefraction in colorectal cancer cells by controlling mucin secretion.*

**12:00** **Short Talk 4. Anna Duarri (VHIR)**.

*Repopulation of decellularized retinas with hiPSC-derived retinal pigment epithelial and ocular progenitor cells shows cell engraftment, organization and differentiation***.**

**12:15** **Short Talk 5. Marta Sureda (IDIBAPS).**

*Sox11 contributes to the stemness and chemoresistance properties via msi2 in aggressive mantle cell lymphoma.*

**12:30** **Aurora Ruiz-Herrera (UAB)**.

*3D genome remodelling in germ cells.*

**13:15** **Lunch, coffee & posters**

**15:15** **Elena Hidalgo (UPF)**.
*Monitoring intracellular $H_2O_2$ fluxes with genetically-encoded reporters.*

**16:00** **Short Talk 6. Ulrich Eckhard (IBMB-CSIC)**.
*Expanding the modularity of (proteolytic) flagellins to generate versatile superhero bugs*.

**16:15** **Short Talk 7. Alejandro Cruz (UAB).**
*How Reticulocyte 15-Lipoxygenase-1 catalyzes the Lipoxin Formation in the 5(S),15(S)-DiHpETE Biosynthetic Pathway? A Theoretical Determination of the Molecular Details of its Mechanism.*

**16:30** **Short Talk 8. Jaime Santos (IBB-UAB)**.
*α-Helical peptidic scaffolds to target α-synuclein toxic species with nanomolar affinity*.

**16:45** **Oscar Llorca (CNIO).**
*Using cryo-electron microscopy to study molecular mechanisms in cancer*.

**17:30** **Beer and poster/short talk prizes**

**18:30** **End**

# PRESENTACIONS

## 1.

**Crosstalk between CK2 & phosphoinositides**

Laura Pastor Gonzalez

Institut de Biologia Molecular de Barcelona (IBMB-CSIC)

Casein Kinase 2 (CK2) is a constitutively active, ubiquitous enzyme which seems to play a universal role sustaining cancer progression and cell proliferation. During years, CK2 was thought to exists as a tetrameric enzyme (tCK2) composed of 2 catalytic and 2 regulatory subunits but recently it has been pointed out the existence of monomeric catalytic subunits (mCK2) related with EMT and cellular stress. However, little is known about the regulation of the tCK2/mCK2 equilibrium and the biological consequences of its alteration. Previous lab research found that disassembly of tCK2 is promoted by its interaction with $PIP_2$, a lipid up-regulated during stress. We have identified the residues contacting the inositol ring and by mutating them we completely obliterated its capacity to bind phosphoinositides. Furthermore, these mutants are also unable to disassembly and form mCK2, as assessed by Native Page. With all these, we propose a model in which phosphoinositides and specifically $PIP_2$ might play a central role controlling the equilibrium between CK2 isoforms.

## 2.

**Polyphosphate degradation by Nudt3-Zn2+ mediates oxidative stress response**

Bàrbara Samper-Martín, Ana Sarrias, Blanca Lázaro, Marta Pérez-Montero, Rosalia Ródriguez-Rodríguez, Mariana PC Ribeiro, Aitor Bañón, Don Wolfgeher, Henning J. Jessen, Berta Alsina, Josep Clotet, Stephen J Kron, Adolfo Saiardi, Javier Jiménez, Samuel Bru

International University of Catalonia (UIC)

Inorganic polyphosphate (polyP) is a polymer of hundreds of phosphate residues present in all organisms. In mammals, polyP is involved in crucial physiological processes, including coagulation, inflammation, and cellular stress response. However, even after decades of research, the enzymatic activities responsible for its metabolism are still unknown. Here, we biochemically purify the polyP phosphatase activity present in rat brain. We identify in Nudt3, a protein from the NUDIX family, the enzyme responsible for this activity. We show that Nudt3 shifts its substrate specificity depending on the cation; specifically, Nudt3 is active on inositol pyrophosphates or on polyP depending on the

presence of Mg2+ or Zn2+, respectively. We demonstrate that Nudt3 has in vivo polyP phosphatase activity in human cells. We show that cells with altered polyP levels by modifying Nudt3 protein amount, present reduced viability upon oxidative stress and increased DNA damage, suggesting that polyP and Nudt3 play a role in oxidative stress response. Finally, we show that Nudt3 is involved in the early stages of the embryo development in zebrafish. This work, by elucidating the original Zn2+-dependent polyP phosphatase activity of Nudt3, represents a long awaited discovery that opens the door for expanding our knowledge in this simple but important biological polymer.

## 3.

**Reversing chemorefraction in colorectal cancer cells by controlling mucin secretion.**

Gerard Cantero

Vall d'Hebron Institut de Recerca (VHIR)

15% of colorectal cancers (CRC) cells exhibit a mucin hypersecretory phenotype, which is suggested to provide resistance to immune surveillance and chemotherapy. Our results formally demonstrate that colorectal cancer cells build a barrier to chemotherapeutics by increasing mucins' secretion. We also identify KChIP3, a negative regulator of mucin secretion (Cantero-Recasens *et al.*, 2018), as a risk factor for CRC patients' relapse in subset of untreated tumours. Indeed, cells depleted of KChIP3 are four times more resistant (measured as cell viability and DNA damage) to chemotherapeutics 5-Fluorouracil plus Irinotecan (5-FU+iri.) compared to control cells, whereas KChIP3 overexpressing cells are 10 times more sensitive to chemotherapeutics. Similar increase in tumour cell death is observed upon chemical inhibition of mucin secretion. Finally, we have demonstrated that sensitivity of CRC patient-derived organoids to chemotherapy increases 40-fold upon mucin secretion inhibition. In conclusion, our data show that reducing mucin secretion provides novel means to control chemoresistance of mucinous colorectal cancer cells.

# 4.

## Repopulation of decellularized retinas with hiPSC-derived retinal pigment epithelial and ocular progenitor cells shows cell engraftment, organization and differentiation

Maria Maqueda[1#], Jose Luis Mosquera[1#], José García-Arumí[2], Anna Veiga[3,4], Anna Duarri[2,4*]

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[3] Pluripotent Stem Cell Therapy Group, Regenerative Medicine Program, Institut d'Investigació Biomèdica de Bellvitge – IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.

[4] National Stem Cell Bank-Barcelona Node, Biomolecular and Bioinformatics Resources Platform (PRB2), ISCIII, Madrid, Spain.

The retinal extracellular matrix (ECM) provides architectural support, adhesion and signal guidance that controls retinal development. Decellularization of the ECM affords great potential to tissue engineering; however, how structural retinal ECM affects *in vitro* development, differentiation and maturation of ocular cells remains to be elucidated. Here, mouse and porcine retinas were decellularized and the protein profile analyzed. Acellular retinal ECM (arECM) scaffolds were then repopulated with human iPSC-derived retinal pigment epithelial (RPE) cells or ocular progenitor cells (OPC) to assess their integration, proliferation and organization. 3,837 and 2,612 unique proteins were identified in mouse and porcine arECM, respectively, of which 93 and 116 proteins belong to the matrisome. GO analysis shows that matrisome-related proteins were associated with the extracellular region and cell junction and KEGG pathways related to signalling transduction, nervous and endocrine systems and cell junctions were enriched. Interestingly, mouse and porcine arECMs were successfully repopulated with both RPE and OPC, the latter exhibiting cell lineage-specific clusters. Retinal cells organized into different layers containing well-defined areas with pigmented cells, photoreceptors, Müller glia, astrocytes, and ganglion cells, whereas in other areas, conjunctival/limbal, corneal and lens cells re-arranged in cell-specific self-organized areas. In conclusion, our results demonstrated that decellularization of both mouse and porcine retinas retains common native ECM components that upon cell repopulation could guide similar ocular cell adhesion, migration and organization.

# 5.

**SOX11 contributes to the stemness and chemoresistance properties via msi2 in aggressive mantle cell lymphoma**

Marta Sureda-Gómez[1], Patricia Balsas[1,2], Marta-Leonor Rodríguez[1], Ferran Nadeu[1,2], Anna De Bolós[1], Álvaro Eguileor[1], Marta Kulis[1,2], Giancarlo Castellano[1], Eva Giné[3], Santiago Demajo[1], Iñaki Martín-Subero[1,2], Pedro Jares[1,2,3], Elias Campo[1,2,3], Virginia Amador[1,2]

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3. Hematopathology Section, Department of Anatomic Pathology, Hospital Clínic of Barcelona, University of Barcelona, Barcelona, Spain.

4. Department of Hematology Hospital Clinic of Barcelona, University of Barcelona, Barcelona, Spain.

Mantle cell lymphoma (MCL) is one of the most aggressive mature B cell neoplasm, characterized by the t(11;14)(q13;q32) primary oncogenic event. Two subgroups of the disease with distinct clinical, biological and molecular features have been described. Conventional MCL (cMCL) is the most common subgroup (90%), characterized by lymph node affectation, poor responses to treatment, frequent relapses and short survival. The leukemic non-nodal MCL (nnMCL) subgroup frequently presents peripheral blood (PB) involvement and good prognosis, without requirement of treatment for long time. SRY-related HMG-box gene 11 (SOX11) aberrant overexpression was predominantly found in cMCL, and the lack of its expression has been associated to better prognosis with longer survival in patients with MCL. Lately, several studies have shown the oncogenic role of SOX11 in the pathogenesis of MCL, blocking the B-cell differentiation process, promoting angiogenesis and a protective tumor microenvironment with immune evasive mechanisms.

The pattern of minimal residual disease and consequent relapses observed in cMCL suggest the presence of a cell population resistant to treatments and with higher self-renewal capabilities. Different researches have described the presence of cancer stem cells (CSC) in MCL, characterized by an increase in drug resistance, clonogenic growth, aldehyde dehydrogenase (ALDH) activity and tumorigenicity.

Several studies have highlighted the relevance of SOX11 in self-renewal and differentiation processes in embryonic and progenitor cells. Moreover, it has been demonstrated that SOX11 is *de novo* expressed after reprogramming hematopoietic cells into induced pluripotent stem cells. Accumulating evidences support that SOX11 is expressed in the CSC population of glioblastomas, and that its overexpression enhances CSC properties, increasing ALDH cell activity, mammosphere formation and

drug resistance in mammary cells. Nevertheless, nothing is known about the possible stemness role of SOX11 in MCL.

In the present study, we explored the association between SOX11 overexpression and gene signatures involved in stem cell functions and hematopoietic development, by gene expression analysis, in MCL primary cases, and observed an enrichment of hematopoietic (HSC) and leukemic stem cells (LSC) gene signatures in SOX11+ compared to negative MCLs. Within them, SOX11 directly bound MSI2 promoter, upregulating its expression in vitro. Moreover, different MSI2 intronic enhancers were exclusively activated in SOX11+ MCL cell lines and primary cases. MSI2 RNA-binding protein plays a critical role inhibiting differentiation and promoting proliferation and self-renewal in HSC and different hematological malignancies. MSI2 upregulation significantly associated with poorest survival, independently of other high risk features, in patients with MCL. Knockdown of MSI2 (MSI2KD) in MCL cell lines presented a gene expression profile enriched in proapoptotic- and memory B cell-related genes; whereas HSC and LSC gene signatures were downregulated compared to its control cells (MSI2CT). MSI2KD or inhibition with Ro 08-2750 (Ro) treatments significantly reduced clonogenic growth and increased apoptosis and sensitivity to ibrutinib, in vitro; and reduced the number of MCL-ADLH+ cells from primary peripheral blood samples, in ex vivo experiments. In vivo, MSI2KD presented reduced tumorigenic engraftment into mice bone marrow and spleen, compared to MSI2CT cells.

Overall, our results suggest that SOX11/MSI2 axis might play a key role in sustaining stemness and chemoresistance in MCL, presenting MSI2 as new promising biomarker for MCL-CSC and as potential therapeutic target for relapsed/refractory MCL.

# 6.

**Expanding the modularity of (proteolytic) flagellins to generate versatile superhero bugs.**

Emma Bloch[1], Benjamin G.L. Jenkins[2], Andrew C. Doxey[2], and Ulrich Eckhard[1].

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Bacterial flagellins are the major building block of the flagellar filament, which plays a key role in cell motility, adhesion, and virulence, but also functions as sensory organelle. In 2017 we reported the discovery of the first family of naturally occurring enzymatically active flagellins in more than 100 bacterial species and spanning >35 phylogenetically diverse genera. These flagellins have acquired a unique metallopeptidase domain within their central hypervariable region. We recombinantly expressed and purified flagellinolysin peptidase domains from both Gram negative and positive

bacteria, and demonstrated metal-dependent proteolytic activity. Furthermore, for *Clostridium haemolyticum* we demonstrated activity for purified flagella, and presence of the peptidase domain on the filament via immunogold labelling. Our analysis revealed substrate specificity profiles similar to human matrix metalloproteases (MMPs), suggesting a role in the degradation of extracellular matrix and/or of bacterial biofilm proteins. Notably, an inactivating point mutation within its active site motif HExxH leads to dramatic changes in biofilm phenotype. Importantly, these proteolytic flagellins (flagellinolysins) copolymerize with non-enzymatic structural flagellins and hence transform the bacterial flagellum into a gigantic proteolytic machinery with thousands of enzymatically active sites (*Eckhard et al. 2017, Nat Commun, 8(1):521*, *Eckhard et al. 2020, Sci Rep, 10(1):19052*).

As a *Ramon y Cajal* investigator (2022 to 2027), I know plan to expand this research line to synthetic biology and biotechnology to devise versatile superhero bugs based on flagellar display as improved probiotics, or for e.g. heavy metal sequestering, the efficient enzymatic degradation of plastics, or.

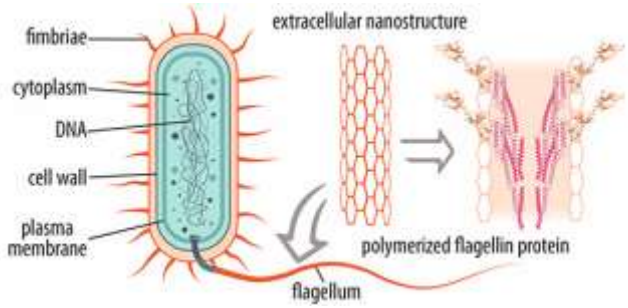


Figure legend. The bacterial flagellum is a highly sophisticated organelle primarily evolved for motility and harbors a long filament consisting of up to 30,000 subunits of the protein building block flagellin. We aim to modify naturally occurring structural flagellins, and augment them with enzymatic functions to create dedicated nanomachines for biotechnology (©Ramon y Cajal Research Group Structural Biotechnology, Ulrich Eckhard, IBMB-CSIC, Barcelona, Spain).

# 7.

**How Reticulocyte 15-Lipoxygenase-1 catalyzes the Lipoxin Formation in the 5(S),15(S)-DiHpETE Biosynthetic Pathway? A Theoretical Determination of the Molecular Details of its Mechanism.**

Alejandro Cruz[1], Àngels González-Lafont[1,2] and José M. Lluch[1,2]

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Chronic inflammation is now widely recognized to play important roles in many commonly occurring diseases, including COVID-19. The resolution response to this chronic inflammation is an active process governed by specialized pro-resolving mediators (SPMs) like the lipid mediators known as lipoxins. The biosynthesis of lipoxins is catalyzed by several lipoxygenases (LOXs) from arachidonic acid. However, the molecular details of the mechanisms involved are not well known yet. In this work, we have combined molecular dynamics (MD) simulations and quantum mechanics/molecular mechanics (QM/MM) calculations to analyze how reticulocyte 15-LOX-1 catalyzes the production of lipoxins from 5(S),15(S)-diHpETE. Our results indicate that the dehydration mechanism from 5(S),15(S)-diHpETE, via the formation of an epoxide, presents huge energy barriers even though it was one of the two a priori synthetic proposals. This result is compatible with the fact that no epoxide has been directly detected as an intermediate in the catalytic formation of lipoxins from 5(S),15(S)-diHpETE. Conversely, the oxygenation of 5(S),15(S)-diHpETE at C14 is feasible because there is an open channel connecting the protein surface with this carbon atom, and the energy barrier for oxygen addition through this channel is small. The analysis of the following steps of this mechanism, leading to the corresponding hydroperoxide at the 15-LOX-1 active site, indicates that the oxygenation mechanism will lead to the formation of lipoxin B4 after the final action of a reductase. In contrast, our calculations are in agreement with experiments that lipoxin A4 cannot derive from 5(S),15(S)-diHpETE by either of the two proposed mechanisms and that 5(S),15(S)-diHETE is not an intermediate of lipoxin biosynthesis catalyzed by 15-LOX-1.

# 8.

## α-Helical peptidic scaffolds to target α-synuclein toxic species with nanomolar affinity

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α-Synuclein aggregation is a key driver of neurodegeneration in Parkinson's disease and related syndromes. Accordingly, obtaining a molecule that targets α-synuclein toxic assemblies with high affinity and conformational selectivity is a long-pursued objective. In this work, we exploit the biophysical properties of toxic oligomers and amyloid fibrils to identify a family of α-helical peptides that bind to these α-synuclein species with low nanomolar affinity, without interfering with the monomeric functional protein. This activity is translated into a remarkable anti-aggregation potency and the ability to abrogate oligomer-induced cell damage.

Using a structure-guided search we also identify a human peptide, LL-37, expressed in the brain and the gastrointestinal tract with analogous binding, anti-aggregation, and detoxifying properties. The identification of LL-37 suggests that this or similar endogenous peptides may be able to interact actively with α-synuclein aggregated species in tissues relevant to the disease.

Overall, the chemical entities we describe here may represent a therapeutic avenue for the synucleinopathies and are promising tools to assist diagnosis by discriminating between native and toxic α-synuclein species. The existence of human peptides with these properties may also open an unexplored avenue for Parkinson's disease treatment, via stimulating their endogenous expression.

# PÒSTERS

## 1.

### 3D chromatin remodeling in the germ line modulates genome evolutionary plasticity

Lucía Álvarez-González[1,2,#], Frances Burden[3,#], Dadakhalandar Doddamani[3,#], Roberto Malinverni[4], Cristina Marín-García[1,2], Laia Marin-Gual[1,2], Albert Gubern[1], Covadonga Vara[1,2], Andreu Paytuví-Gallart[1,2,5], Marcus Buschbeck[5,6], Peter Ellis[3], Marta Farré[3], Aurora Ruiz-Herrera[1,2]

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The spatial folding of chromosomes and their organization in the nucleus has profound regulatory impacts on gene expression and genome architecture, whose evolutionary consequences are far from being understood. Here we explore the evolutionary plasticity of the 3D chromatin remodelling in the germ line given its pivotal role in the transmission of genetic information. Using a comprehensive integrative computational analysis, we (i) reconstruct ancestral rodent genomes analyzing whole-genome sequences of 14 rodent species representatives of the major phylogroups, (ii) detect lineage-specific chromosome rearrangements and (iii) identify the dynamics of the structural and epigenetic properties of evolutionary breakpoint regions throughout mouse spermatogenesis by applying integrative computational analyses. Our results show that evolutionary breakpoint regions are devoid of programmed meiotic DSBs and meiotic cohesins in primary spermatocytes but associated with functional long-range interaction regions and sites of DNA damage in post-meiotic cells. Moreover, we detect the presence of long-range interactions in spermatids that recapitulate ancestral chromosomal configurations. Overall, we propose a model, which integrates evolutionary genome reshuffling with DNA damage response mechanisms and the dynamic spatial genome organization of germ cells.

# 2.

## Use of adenoviral vectors for the delivery of RNA-PolyPurine Reverse Hoogsteen Hairpins

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PolyPurine Reverse Hoogsteen Hairpins (PPRHs) are gene silencing DNA-oligonucleotides developed in our laboratory. They are formed by two antiparallel polypurine mirror repeat domains bound intramolecularly by Hoogsteen bonds, which can hybridize to polypyrimidine targets in the genomic DNA. Among all the technologies developed for nucleic acids delivery, Adenoviruses (AdVs) vectors present high transduction efficiencies on a variety of cells without integrating with the host genome. Since in viral vectors the PPRH sequence would be transcribed into RNA, we first evaluated the effect of a PPRH targeting survivin as an RNA species (synthetic RNA-PPRH). We demonstrated the ability of the RNA-PPRH to bind to its target sequence, either ssDNA or dsDNA. We showed that the RNA-PPRH induced a decrease on cell viability in a dose-dependent manner and an increase of apoptosis in PC-3 and HeLa cells. We also determined that both the synthetic RNA-PPRH and an RNA-PPRH intracellularly generated upon transfection of a plasmid vector were able to reduce survivin mRNA and protein levels of PC-3 cells. Once validated that RNA-PPRHs induced survivin silencing, we confirmed that an AdV5 encoding a PPRH against survivin was able to decrease survivin mRNA and protein levels, leading to a reduction in HeLa cell viability. In contrast, no effect was observed in cells infected with the negative control AdV-GFP. In this work we demonstrated that PPRHs can also work as RNA species, using RNA chemically synthesized, plasmid and viral expression vector. Therefore, we proved that adenoviral vectors can be considered as a delivery system of PPRH.

# 3.

**Mining drug-target and drug-adverse drug reactions databases to identify target-adverse drug reactions relationships.**

Cristiano Galletti

Universitat Central de Catalunya

The level of attrition on drug discovery, particularly at advanced stages, is very high due to unexpected adverse drug reactions (ADR) caused by drug candidates, and thus, being able to predict undesirable responses when modulating certain protein targets would contribute to the development of safer drugs and have important economic implications. On the one hand, there are a number of databases that compile information of drug-target interactions. On the other hand, there are a number of public resources that compile information on drugs and ADR. It is therefore possible to link target and ADRs using drug entities as connecting elements. Here we present T - ARDIS (Target - Adverse Reaction Database Integrated Search) database, a resource that provides comprehensive information on proteins and associated ADRs. By combining the information from drug-proteins and drug-ADR databases, we statistically identify significant associations between proteins and ADRs. Besides describing the relationship between proteins and ADRs, T-ARDIS provides detailed description about proteins along with the drug and adverse reaction information. Currently T-ARDIS contains over 3000 ADR and 248 targets for a total of more 17000 pairwise interactions. Each entry can be retrieved through multiple search terms including target ID, target Gene name, adverse effect and Drug name. Ultimately, T-ARDIS database has been created in response to the increasing interest in identifying early in the drug development pipeline potentially problematic protein targets whose modulation could result in ADR.
Database URL: . http://www.bioinsilico.org/T-ARDIS

## 4.

**Strategies to solve aggregation of a mitochondrial membrane protein**

Illanes-Vicioso, Ramiro; Ruiz-López, Elena; Solà, Maria.

Institut de Biologia Molecular de Barcelona (IBMB-CSIC)

We are interested in a mitochondrial transmembrane protein located at the inner membrane. The protein consists of three domains of unknown function. The N- terminal domain is found at the intermembrane space, a transmembrane helix crosses the inner membrane, and it is followed by the C-terminal domain at the mitochondrial matrix. Several constructs, about thirty, were tested in order to generate the most stable protein fragment, suitable for expression and crystallization. Even so, very few genetic constructs, all derived from the N- terminal domain, were soluble. Only one construct, N4, showed good expression levels in *Escherichia coli* and could be isolated. Nevertheless, N4 had a high tendency to aggregate, suggesting its stability was not enough. We will present the different purification steps and the re-thinking of the construct to overcome these problems and find the key to reach protein stability and production in high yields.

## 5.

**Multi-ocular Organoids from Human iPS Cells Displayed Retina, Cornea, and RPE Lineages**

Helena Isla

Vall d'Hebron Institut de Recerca (VHIR)

The mammalian eye is a complex organ, comprising different highly specialized tissues derived from various cell linages, including neural ectoderm, surface ectoderm, neural crest and the periocular mesenchyme. Great efforts have been made to design protocols for obtaining ocular cells from human stem cells to model diseases or for regenerative purposes, but the complex crosstalk between ocular cell types driving self-organizing growth is usually limited by restricted experimental designs. Current protocols are overall focused on the isolation of retinal, retinal pigment epithelium (RPE) or corneal cells. Here, we obtained multi-ocular organoids from human induced pluripotent stem cells. First, self-formed multi-zone ocular progenitors in 2-dimensional culture spanned the neuroectoderm, surface ectoderm, neural crest and RPE. After manual isolation and growth in suspension, they developed into different 3D multi-ocular organoids composed of multiple cell lineages: retinal pigment epithelium, retina and cornea; which could be generated also individually. Within these organoids, retinal regions display correct layering and harbor all major retinal cell subtypes as well as retinal morphological cues, whereas corneal regions closely resemble the transparent ocular-surface epithelium with characteristics

of corneal, limbal and conjunctival epithelial cells. RPE also arranged to form organoids composed of polarized pigmented epithelial cells at the surface, full-filled with collagen matrix. These multi-ocular organoids offer a new platform to study early human eye development and disease, and provide a source of human ocular cells from the same individual.

# 6.

**Standardization of *in vitro* models of endothelial cell dysfunction by induced senescence.**

Francisco Rafael Jimenez-Trinidad, Marta Arrieta, Núria Solanes, Angela Vea, Joaquim Bobi, Antoni Valera, Mercè Roqué, Xavier Freixa, Manel Sabaté, Ana Paula Dantas, Olga Tura-Ceide, Montserrat Rigol.

Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)

Background: Endothelial cell dysfunction is the principal cause of several cardiovascular diseases that are increasing in prevalence, healthcare costs and mortality. Developing a standardised, representative *in vitro* model of endothelial cell dysfunction is fundamental to a greater understanding the pathophysiology and aid development of novel pharmacological therapies.

Methods: We subjected human umbilical vein endothelial cells (HUVECs) to different periods of nutrient deprivation or increasing doses of $H_2O_2$ to represent starvation or elevated oxidative stress, respectively, to investigate changes in cellular functional.

Results: HUVECs showed a significant $H_2O_2$ concentration dependent reduction in cell viability ($p<0.0001$) and a significant increase in oxidative stress ($p<0.0001$). Furthermore, HUVECs subjected to 96h of starvation or exposed to concentrations of $H_2O_2$ of 400 to 1000μM resulted in decreased cellular function. Functional alterations included a reduction in angiogenic and migratory capabilities of the cells and an increase in β-galactosidase activity and senescence gene markers (SIRT1, P21 and TP53). Both *in vitro* cellular models of endothelial cell injury developed in this study, starvation and oxidative stress, were validated by markers of cellular senescence and endothelial dysfunction.

Conclusion: These models will enable improved physiological studies of endothelial cell dysfunction and the rapid testing of cellular efficacy and toxicity of future novel therapeutic compounds.

Funding

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# 7.

**Structural basis for the SUMO protease activity of USPL1, an atypical ubiquitin USP family member**

Ying Li, Nathalia Varejão and David Reverter

Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular. Universitat Autònoma de Barcelona, Bellaterra, Spain.

Post-translational protein modification by ubiquitin and ubiquitin-like modifiers regulate all major pathways in the cell, such as cell cycle control, protein homeostasis or DNA-repair, and their action can be reversed by the action of specific proteases called de-ubiquitinases. USPs constitute the largest family of de-ubiquitinases in cells having in common their proteolytic activity towards ubiquitin, except for USPL1, which is a distant member the USP family with a unique preference for SUMO. Here, we present the crystal structure of the complex between USPL1 and SUMO2 and we disclose the key structural elements for such unusual deSUMOylase activity of a USP family member. In addition to specific contacts between USPL1 and SUMO2, such as the unique hydrogen bond network of the C-terminal tail with the active site groove of USPL1, a major structural element common in all ubiquitin USPs, the "Blocking Loop", is missing in USPL1, facilitating SUMO binding. This is a paradigmatic example of divergent evolution in the USP family, where a structural protein scaffold designed to bind ubiquitin has evolved in USPL1 to bind SUMO, a distant UbL modifier.

# 8.

**Revealing the role of ATPase TRIP13 in synaptonemal complex formation**

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Meiosis is the reductional cell division that produces haploid cells – gametes – from a diploid progenitor. This process consists of a single round of DNA replication followed by two rounds of cell divisions. Aneuploidies, such as Down syndrome, which are the leading cause of birth defects and

developmental disabilities in humans, are due to failures in this process. The ATPase TRIP13, also known as Pch2 in non-vertebrate species, is involved in several processes crucial for cell cycle progression, such as chromosome segregation, chromosome axis formation, and DNA repair. Previous studies performed in mammals have shown that TRIP13 is required for proper homologous chromosome synapsis. However, it is still unclear how TRIP13 mediates synapsis in mouse spermatocytes.

To learn more insights about this and other TRIP13 meiotic functions, we immunoprecipitated TRIP13 from mouse testis protein extracts and identified its interacting partners by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results comparing wild-type and *Trip13*$^{-/-}$ mutant samples revealed that the chaperone HSPA2 is one of the strongest interactors of TRIP13 in the testis. It had been previously described that HSPA2 is required to disassemble the synaptonemal complex (SC) during diplonema. The synaptonemal complex is a highly conserved meiosis-specific structure that maintains homolog chromosomes together and ensures that each gamete receives one copy of each chromosome. Interestingly, HSPA2 location in mouse spermatocytes is dependent on TRIP13, so that *Trip13*$^{-/-}$ spermatocytes present a precocious loading of HSPA2 in pachytene spermatocytes. Accordingly, *Trip13*$^{-/-}$ spermatocytes present fewer SC initiation sites, as it is shown in SYCP1 labeled chromosome spreads, and premature loading of other desynapsis factors, like PLK1. Hence, we propose a model in which TRIP13 is responsible to prevent precocious SC disassembly at the pachytene stage by regulating HSPA2 presence in the chromatin of mouse meiocytes.

# 9.

**eEF1A2 controls translation and actin dynamics in dendritic spines**

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Synaptic plasticity involves structural modifications in dendritic spines that are modulated by local protein synthesis and actin remodeling. Here, we investigated the molecular mechanisms that connect synaptic stimulation to these processes. We found that the phosphorylation of isoform-specific sites in eEF1A2—an essential translation elongation factor in neurons—is a key modulator of structural plasticity in dendritic spines. Expression of a nonphosphorylatable eEF1A2 mutant stimulated mRNA translation but reduced actin dynamics and spine density. By contrast, a phosphomimetic eEF1A2 mutant exhibited decreased association with F-actin and was inactive as a translation elongation factor. Activation of metabotropic glutamate receptor signaling triggered transient dissociation of eEF1A2

from its regulatory guanine exchange factor (GEF) protein in dendritic spines in a phosphorylation-dependent manner. We propose that eEF1A2 establishes a cross-talk mechanism that coordinates translation and actin dynamics during spine remodeling.

## 10.

**Polypurine Reverse Hoogsteen hairpins as a tool to promote exon skipping at the genomic level**

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Exon skipping strategies are aimed to mediate the elimination of mutated exons containing premature stop codons and to restore the reading frame of the affected protein. We explored the capability of polypurine reverse Hoogsteen hairpins (PPRHs) to cause exon skipping at the genomic level in a cell line carrying a *dihydrofolate reductase (dhfr)* minigene pD22, with a duplicated exon 2 that causes a frameshift.

Three PPRH target regions were identified in the pD22 minigene, localized in the promoter, exon 3 and exon 6, respectively. Each PPRH core was extended at its 5' end by a sequence tail homologous to 20 nt upstream and/or 20 nt downstream of a PstI restriction site, which corresponded to the insertion site of the additional exon 2 in the pD22 minigene. The different Editing-PPRHs were transfected followed by cell incubation in DHFR selective medium lacking hypoxanthine and thymidine. PPRHs carrying the whole homologous tail including both the 20 nt upstream and downstream the Pst I site originated colonies in selective medium. DNA sequencing results proved that the *dhfr* sequence in these cells corresponded to the wild type with just one copy of exon 2. The skipping of the additional exon 2 was confirmed at the mRNA level, DHFR protein was restored, and it showed high levels of DHFR activity.

We conclude that editing-PPRHs are able to cause exon skipping at the DNA level and could be applied as a possible therapeutic tool for genetic diseases such as Duchenne muscular dystrophy.

## 11.

**Purification strategies to isolate a highly flexible DNA binding protein prone to degradation**

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We are working on a DNA-binding protein that bears an HMG-box domain accompanied by large disordered regions, which probably become structured upon ligand binding. In general terms, intrinsically disordered regions are sticky and are not easy to purify. Our collaborators sent us the protein gene, which we fused to pCRI7b vector that adds a 6-histidine tag to the C-terminal end. We performed expression tests in E. coli BL21(DE3) strain, which showed an extra thin expression band compared to the non-expressing control sample. The following solubility tests with this sample showed that the protein was soluble at pH 7, 9 and 11, in the presence of 250, 500 or 1000 mM NaCl. With the best solubilization buffer, we performed several IMAC purifications with a Nickel-chelating column at different pHs to tune the binding affinity of the protein and improve protein purification. With a buffer that showed the best balance between protein expression and isolation, we did extensive stepwise chromatographies to check at which imidazole concentration the protein purity was highest. From this, we could design an optimal three-step IMAC allowing us to quickly produce pure protein. During all these procedures, the protein suffered important degradation thus we also explored strategies to solve this problem. We will show the procedures and tricks to optimize the quality of the sample obtained.

## 12.

**Differential autophagy in Drosophila larval trachea**

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Autophagy is a lysosome-mediated cellular degradation process that can either contribute to cell survival by recycling its own constituents or to cell death at elevated levels. This process has been showing their implications in multiple roles as tissue degradation, renovation and development among others.

During the metamorphosis of Drosophila melanogaster, driven by the moulting hormone Ecdysone, the juvenile tissues have to be removed before the adult structures are formed. To carry out this process, most of larval cells die along this stage, while others, the Adult Progenitor Cells (APCs), survive and give rise to the adult organism.

It has already been shown that larval cells fated to die use autophagy as a preparative phase of programmed cell death to free up stored biomass that is utilized by the APCs as a nutrient and energy source during metamorphosis (Lörincz et al. 2017). In this stage of Drosophila's development, autophagy has been found to have a role in the degradation of structures such as the larval midgut, the fat body and the salivary glands (Berry and Baehrecke 2007, Denton et al. 2009).

In this project, we study the specific role of autophagy in the Drosophila larval tracheal system. This organ is particularly interesting to examine this process, because it´s compose of some different kind of cells, which have to react in a different way to the metamorphosis signals, as developing or dying. There are polyploid cells, fated to die at metamorphosis, and diploid ones, that are progenitors of the adult tracheal system and therefore, have to survive.

In fact, our results show that autophagy is differentially active in the tracheal segments made up of polyploid cells versus the one that consists of APCs.

Now, we are working in analyse how the perturbation of this differential expression can affect the trachea degeneration in the pupal stage, and if this is affecting also the viability of those animals.

## 13.

**Structural insights into MraZ conformation and DNA Binding**

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Cell division is a fundamental cellular process and the basis of microorganism survival. The molecular machinery involved in cell division is then exposed to a strong evolutive pressure that in bacteria results in highly conserved gene division clusters and regulatory proteins. MraZ is a small transcription factor present in many bacteria, which is involved in cell division by regulating the *division cell wall (dcw)* gene cluster. Whereas the promotor recognition sequence of MraZ is well-known in many organisms, the molecular determinants driving MraZ binding to DNA remain to be elucidated. Here, we study MraZ protein of the model organism *Mycoplasma genitalium* to obtain structural insights into its mode of action and mechanism of DNA interaction. To date, we have solved MraZ structure without DNA and obtained two different conformations. One form is an octameric ring, highly similar to the structure previously determined in its close relative, *Mycoplasma pneumoniae*. However, we also discovered that MraZ also forms a nonameric assembly under some crystallization conditions.

Both *Mycoplasma genitalium* and *Mycoplasma pneumoniae* are considered minimal cells and thus their biological processes and proteins are subjected to a substantial evolutionary optimization. Currently, we aim to find if these two oligomeric states are biologically relevant and associated with function. To that aim, we are working on the obtention of MraZ-DNA complex structure via crystallization methods. Overall, we expect that exploring MraZ oligomerization and DNA binding may not only shed light into MraZ mechanistic details but also help us elucidate the intricate regulatory network behind *dcw* cluster.

## 14.

**Effect of Polypurine Reverse Hoogsteen Hairpins on G-quadruplexes of *c-MYC* and *K-RAS* oncogenes in different cancer cell lines**

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It has been proved that G-quadruplex (G4s) are involved in the transcriptional and translational regulation of cellular genes. Previous results of our laboratory demonstrated that targeting the complementary strand of G4 forming sequences (G4FS) in the 5'-UTR of the Thymidylate synthase (TYMS) was very effective in reducing the viability, mRNA and protein levels of this target. In this report, we used PolyPurine Reverse Hoogsteen hairpins (PPRHs) against polypyrimidine segments in the genes coding for either *c-MYC* or *K-RAS* complementary to G4FS to promote G4 folding thus increasing gene silencing. PPRHs hairpins are polypurine strands running in antiparallel orientations, bound by intramolecular Hoogsteen bonds and linked by a thymidine loop. These molecules bind to their polypyrimidine target region of DNA or RNA by Watson-Crick bonds and provoke a strand displacement. First, we searched putative targets of PPRHs in *c-MYC* and *K-RAS* genes using the Triplex-forming Oligonucleotide Target Sequence Search software. Then, we selected those forming G4 structures *in silico* using the Quadruplex forming G-Rich Sequences (QGRS) mapper. Subsequently, we designed the corresponding PPRHs against the two target genes. PPRHs were transfected with the cationic liposome Dioleoyl-Pyridinium (DOPY) and reduced the cell viability of neuroblastoma, prostate, breast, and cervix cancer cell lines. The most effective PPRHs against the promoter of *K-RAS* was HpKRAS-PR-C in all the cell lines tested. In the case of c-*MYC* it was HpMYC-I1-WT-T directed against intron 1 showed a decrease higher than 95% of viability in prostate cancer cells. We also obtained a decrease in mRNA levels of *c-MYC* and *K-RAS* genes in prostate cancer cell line. Additionally, in the MiaPaCa pancreas cancer cell line, we tested *K-RAS* PPRHs transfected with the cationic liposome Dioleoyl-3-trimethylammonium propane (DOTAP) obtaining a

significant decrease in cell viability. Our results show that the G4 regions are good targets for PPRHs to provoke specific oncogene gene-silencing and therefore be used for cancer treatment.

## 15.

**Structural basis for the E3 ligase enhancement of yeast Nse2 by SUMO- Interacting Motifs**

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Post-translational modification of proteins by ubiquitin and ubiquitin-like modifiers, such as SUMO, are key events to regulate cellular processes such as protein homeostasis or DNA damage response. Smc5/6 is a nuclear multi-subunit complex that participates in the recombinational DNA repair processes and is required in the maintenance of chromosome integrity. Nse2 is a subunit of the Smc5/6 complex that possesses SUMO E3 ligase activity by the presence of a SP- RING domain that activates the E2-SUMO thioester for discharge on the substrate. Here we have solved the crystal structure of the SUMO E3 ligase, Nse2/Smc5, in complex with an E2-SUMO thioester mimetic. In addition to the interface between the SP-RING domain and Ubc9, the complex reveals other interfaces in non-SP-RING elements that are essential for the E3 ligase activity. In particular, two SIM-like motifs in Nse2, SIM1, and SIM2, are restructure upon binding the donor SUMO and the E2 backside SUMO during the E3-dependent discharge reaction, and both SIM interfaces are essential in the conjugation activity of Nse2 and are required to cope with DNA damage.