

# La SEBBM i la bioquímica valenciana 1963-2023

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Sala Darwin, Campus Burjassot  
Universitat de València



SEBBM  
SEBBM

***Organización:***

- Sociedad Española de Bioquímica y Biología Molecular (SEBBM)
- Departamento de Bioquímica y Biología Molecular. Universidad de Valencia

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# LA SEBBM Y LA BIOQUÍMICA VALENCIANA

## 1963-2023



La Sociedad de Bioquímica y Biología Molecular (SEBBM) organiza una jornada científica para conmemorar el 60 aniversario de la SEBBM (1963-2023). En esta jornada, con formato de registro gratuito, se presentará la SEBBM como una de las sociedades más relevantes del sistema de ciencia español y al mismo tiempo queremos dar la oportunidad a nuestros estudiantes de bioquímica e investigadores más jóvenes de tener una visión, tanto de lo que se realiza en algunos laboratorios de Valencia, como ofrecerles un foro donde presentan en formato póster (DIN A0 vertical) sus propios trabajos de investigación en el área de la Bioquímica y la Biología Molecular (TFGs, TFM, resultados preliminares de sus proyectos de tesis...). De los pósters presentados se seleccionará uno como ganador del Premio 60 años SEBBM Valencia a la mejor comunicación.

# ORGANIZACIÓN



Ismael Mingarro



Mar Orzaez



Mª Jesús García

# PONENCIAS

Hora	Sesión	
9.15	Presentación a cargo de la Presidenta de la SEBBM Isabel Varela-Nieto.	
9.30	Antonio Ferrer-Montiel (IDiBE-UMH) Apoyo a la Ciencia traslacional y transferencia tecnológica en la SEBBM.	
10.00	Puri Lisón (IBMCP-UPV) El aroma de la resistencia.	

Hora	Sesión
10.30	<p><b>Marçal Vilar (IBV-CSIC)</b>  <b>Presenilins, A<math>\beta</math> and p75, a matter of three in cholinergic neurodegeneration.</b></p> 
11.00	<p><b>Pòsters amb cafè.</b>  <b>Sessió de pòsters coordinada per Jerónimo Bravo (IBV-CSIC).</b></p> 
12.30	<p><b>Patricia Casino (BIOTECMED-UV)</b>  <b>Structural and functional bases of microbial signal transduction systems.</b></p> 
13.00	<p><b>M<sup>a</sup> Àngels Juanes (CIPF)</b>  <b>Cytoskeletal dynamics in cell migration and invasion.</b></p> 
13.30	<p><b>Vicente Rubio (IBV-CSIC)</b>  <b>Recapitulando 60 años de vida de SEBBM (y anticipando otros tantos).</b></p> 
14.00	<p><b>Entrega del Premio al mejor Póster y Clausura de la Jornada.</b></p>

# PÓSTERS

## Póster 1

"María Aguilar-Ballester (1); Elena Jiménez-Martí (1,2,3); Gema Hurtado-Genovés (1); Alida Taberner-Cortés (1); Ángela Vinué (1); Andrea Herrero-Cervera (1); Sergio Martínez-Hervás (1,4,5); Herminia González-Navarro (1,2,5).

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### **Estudio del papel de la citoquina LIGHT sobre las subpoblaciones inmunes en un modelo murino de EHGNA.**

La enfermedad del hígado graso no alcohólica (EHGNA) es una de las principales complicaciones metabólicas de la diabetes mellitus tipo 2. La inflamación hepática induce la transición a la esteatohepatitis no alcohólica (EHNA), una condición metabólica disfuncional hepática caracterizada por fibrosis que podría terminar en hepatocarcinoma celular (HCC). Algunos estudios sugieren efectos divergentes de los sublinajes hepáticos de las células T naturales asesinas (células NKT, del inglés natural killer T cells), en la modulación de la progresión de EHGNA-EHNA-HCC. Estudios previos nuestros han demostrado que la deficiencia de la citoquina LIGHT disminuye la expresión de Plzf, un factor clave en la diferenciación de las células NKT, lo que sugiere que LIGHT podría modular estas poblaciones de células inmunes hepáticas durante la progresión de la enfermedad hacia EHNA. En el presente estudio se ha realizado la caracterización de las diferentes células NKT intrahepáticas y un posible papel de LIGHT en un modelo murino de EHNA inducido por dieta.

#### **Materiales y métodos:**

Los ratones Wt y Light-/- recibieron una dieta control (DC) o una dieta rica en azúcares, grasa y colesterol (DEHNA) durante 16 semanas. Después de la dieta, se determinó el porcentaje del área de colágeno con tinción tricrómica de Masson y la puntuación NAS en secciones transversales hepáticas. Además, se realizaron análisis de leucocitos circulantes y subpoblaciones de linfocitos, incluidas las células NKTs, y monocitos intrahepáticos mediante citometría de flujo los 4 grupos de ratones.

#### **Resultados:**

Como era de esperar la puntuación NAS y el contenido de colágeno aumentó en los ratones Light-/- y Wt alimentados con DEHNA en comparación con sus homólogos alimentados con DC. Sin embargo, el contenido de colágeno en los ratones Light-/- alimentados con DEHNA se redujo significativamente en comparación con los ratones Wt. El análisis de células

inmunitarias intrahepáticas mostró un contenido reducido de macrófagos antiinflamatorios F4/80+Cd206c+ en ratones Light-/- y Wt alimentados con DEHNA consistente con un estado inflamatorio reducido. El examen de linfocitos hepáticos mostró un aumento en las células NKT17 proinflamatorias en ratones Wt tratados con DEHNA pero no en los ratones Light-/-.

### **Conclusiones:**

La deficiencia de Light reduce el contenido de colágeno en ratones alimentados con DEHNA, lo que podría indicar un estado de fibrosis reducido. Además, los cambios observados en las poblaciones de células inmunes intrahepáticas sugieren un papel de esta citoquina en la diferenciación de las células NKT durante la progresión de EHNA a EHNA.

### **Póster 2**

**Sonia Albillos-Arenal (1), Amparo Querol (1) and Eladio Barrioa (2)**

(1) IATA-CSIC. Paterna, Spain; (2) Departament of Genetics, Universitat de València, Spain.

### **Deciphering the pathway of erythritol synthesis in *Saccharomyces* species and its function.**

During wine fermentation, the cryotolerant *Saccharomyces uvarum* differs from its counterpart *S. cerevisiae* in the amount and timing of the production of fermentative by-products, including erythritol. Erythritol pathway has never been reported in *Saccharomyces* genus, although it has been described in other yeasts, which involves the reduction of erythrose into erythritol by erythrose reductases (ERs). The genes encoding for the ERs involved in this reaction have been detailed in the osmotolerant yeast species *Yarrowia lipolytica*.

We used phylogenetic and genetic comparative approaches to identify putative ERs in *Saccharomyces* species, and the genes GCY1, YPR1, ARA1, GRE3, and YJR096W were selected. These genes were knocked out in BMV58, a *S. uvarum* strain that produced a valuable amount of erythritol. Then, fermentation was performed by BMV58 and the single and combined mutants, and the production of erythritol and other metabolites was measured. Our study found that the single deletion of GRE3 significantly decreased erythritol production, indicating that this gene encodes the main enzyme involved in erythritol production. Nevertheless, other genes could also be involved in the synthesis of erythritol as all the knockout combinations decreased erythritol production, especially the triple knockout mutant  $\Delta$ YPR1 $\Delta$ GCY1 $\Delta$ GRE3.

Erythritol production could have a double function. It could be produced as an osmoprotectant against different stresses, which is consistent with the role observed in other yeasts. Besides, it could be involved in redox homeostasis and aid in the reoxidation of NADPH to balance the cofactors produced by the synthesis of succinate at the GABA shunt level.

**Póster 3**

Andrea Alcántara-Enguídanos (1,2); Sara Rosa-Téllez (1,2); Roc Ros (1,2)

(1) Departamento de Biología Vegetal, Universitat de València, Burjassot, Spain; (2) Instituto Universitario de Biotecnología y Biomedicina (BIOTECMED), Universitat de València, Burjassot, Spain

**Serine metabolism reprogramming to tackle biotic stresses under climate change conditions in *Arabidopsis***

Climate change is causing more extreme weather patterns that open new challenges for agriculture due to biotic and abiotic stresses. In this context, metabolic reprogramming is one of the plant's mechanisms for responding to environmental stresses, with L-Serine (Ser) being one of the metabolites that is constantly increasing in response to many of them (Obata et al., 2015; Karmakar et al., 2019; Rosa-Téllez et al., 2019). Ser is the precursor of some metabolites that play a crucial role in the plant response to biotic stresses, such as glucosinolates (GSL), and whose biosynthesis mechanisms are altered by climate change conditions. In the Brassiceae plant family, which includes species such as broccoli, some metabolites derived from the hydrolysis of GSL (isothiocyanates) have been shown to have bioactive properties related to cancer prevention, as well as in plant defense mechanisms against pathogens and insects. Under elevated CO<sub>2</sub> conditions (eCO<sub>2</sub>) the main Ser biosynthesis pathway, known as the glycolate pathway (GPSB) (Cascales-Miñana et al., 2013; Toujani et al., 2013; Ros et al., 2014), is reduced. Thus, when GPSB is limited by eCO<sub>2</sub> concentrations, a Ser reduction occurs which compromise the ability of plants to cope with biotic/abiotic stresses. Under these conditions, in which Ser supply by GPSB is limited the phosphorylated pathway of Ser biosynthesis (PPSB) is induced.

As a first attempt, we checked the GSL content under different CO<sub>2</sub> concentrations in both aerial part (AP) and roots tissues, in Wild-type (WT) and overexpression lines of the phosphoglycerate dehydrogenase 1 isoform (OexPGDH1) that perform the first step of the PPSB. Our results indicate that under eCO<sub>2</sub> there is an increase in GSL content in both WT and OexPGDH1 lines. Notwithstanding, major changes were observed in the roots of OexPGDH1 lines when plants were transferred from ambient to eCO<sub>2</sub>.

To undertake in-depth study of PPSB role in GSL biosynthesis under climate change conditions, we obtain transgenic lines overexpressing target genes of PPSB and GSL biosynthetic pathway in order to further investigate the metabolic networks connecting PPSB and the capacity to respond to biotic/abiotic stresses under climate change conditions. We expect that this work will lead to an improvement of the defense mechanism of plants against pathogen attack under climate change conditions, which may be favourable for reducing pesticide use in the future, in line with the objective of the Sustainable Development Goals of the ONU 2030 agenda.

**Póster 4**

A. Andrés-Bordería (1); A. Perea-Garcia (2); L. Peñarrubia (2); S. Puig (1)

(1) CSIC-IATA; (2) Universitat de València

### **Characterization of a truncated copper transporter from *Saccharomyces cerevisiae***

Copper is essential for processes such as the electron transport chain at the mitochondrion. At the same time, its ability to donate and accept electrons makes it toxic by producing reactive oxygen species. For this reason, the intracellular copper concentration is tightly regulated through the activation and repression of genes encoding proteins involved copper homeostasis components. These components are involved in the copper acquisition, compartmentalization, and chelation, which allow copper uptake while avoiding its toxic effects. Copper uptake into the yeast *Saccharomyces cerevisiae* mainly occurs through the high-affinity copper transporters Ctr1 and Ctr3. These transporters are proteins with three transmembrane domains and their amino-terminal end facing outside the cell and their carboxy-terminal end toward the cytosol. The carboxyl-terminal end of the Ctr1 transporter of this yeast is longer than in other species and is rich in methionine and cysteine residues.

In order to deregulate copper uptake in *S. cerevisiae*, we have obtained a construct with the Ctr1 transporter with the first 300 amino acids, lacking the carboxyl-terminal end [CTR1(300)]. Previous studies have shown that the carboxyl-terminal end of yeast Ctr1 regulates copper uptake, since its loss results in increased copper accumulation and the subsequent increased sensitivity to copper excess. Our data confirms that the strain with the truncated transporter shows a higher copper influx but also an increased iron content. This altered metal content redounds in the increased expression of the copper detoxification genes, such as the metallothioneins CUP1 and CRS5, as well as the iron uptake genes FTR1 and FET3. Our data also suggest that iron-sulfur clusters proteins could be damaged in the CTR1(300) strain. Although the loss of cell viability prevents the measure of the increased oxidative damage produced by copper, different CTR1(300) phenotypes have been observed that could be explained by the damage produced by copper at the iron-sulfur clusters proteins. Due to the interest of yeast in the agri-food industries, increased iron content in strain CTR1(300) could be considered an attractive feature for future biotechnological strategies.

**Póster 5**

Marina Barba-Aliaga (1,2); Brian M. Zid (3) and Paula Alepuz (1,2).

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## Translation factor eIF5A modulates the mitochondrial activity through the control of mitochondrial protein import.

Eukaryotic initiation translation factor eIF5A is an essential and highly conserved protein across archaea and eukaryotes. It is the only known protein containing the post-translational modification hypusine, essential for its activity. Hypusinated eIF5A binds ribosomes to facilitate translation of motifs with polyprolines or combinations of proline with glycine and charged amino acids. eIF5A is linked to other molecular functions and cellular processes such as nuclear mRNA export, proliferation and apoptosis. Moreover, eIF5A has been linked to the pathogenesis of several diseases, such as cancer, diabetes or neurological diseases and ageing. Our lab and others have found that eIF5A is essential for the mitochondrial activity, thus its depletion reduces mitochondrial respiration and membrane potential. Previously, we have demonstrated how eIF5A expression responds to the metabolic state of *Saccharomyces cerevisiae*. Positive and negative regulation of eIF5A is controlled by the transcription factor Hap1, which upregulates the respiratory isoform of eIF5A under respiratory conditions.

Recently, we have identified an essential mitochondrial protein as a direct eIF5A target for translation: the mitochondrial inner membrane protein Tim50. Tim50 is part of the TIM23 complex, which is involved in the import of most mitochondrial proteins targeting the mitochondrial inner membrane and the matrix. Tim50 amino acid sequence contains consecutive proline stretches requiring eIF5A for its synthesis. Upon eIF5A depletion, Tim50 translation stalls in the mitochondrial surface and its protein levels decrease. Thus, the import of proteins into mitochondria is reduced as well. Consequently, the mitoCPR stress response is triggered to promote the removal of accumulated proteins in the mitochondrial surface but it is also observed the accumulation of mitoproteins cytosolic aggregates which cannot reach its functional location. Overall, these results explain why eIF5A is essential for the mitochondrial function.

### Póster 6

Lautaro Baro(1,2,3), Asifa Islam(1,2), Hanna Brown(1,2), Zoe Bell(1,2) and M. Angeles Juanes(1,2,3)

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## APC-driven actin nucleation controls collective cell remodelling and motility in colorectal cancer cells

Cell remodeling relies on dynamic rearrangements of cell contacts powered by the actin cytoskeleton. The tumor suppressor Adenomatous Polyposis Coli (APC) nucleates actin filaments (F-actin) [1-3] and localizes at cell junctions [4,5]. Whether APC-driven actin nucleation acts in cell junction remodeling remains unknown. To explore that possibility, we have combined bioimaging and genetic tools with artificial intelligence algorithms applied to

colorectal cancer cell monolayers. We found that the APC-dependent actin pool contributes to sustaining levels of F-actin and adhesive components at cell junctions. Moreover, this activity preserved cell junction length, angle and motion, as well as vertex motion and integrity. Finally, the loss of this F-actin pool led to larger cells with slow and random cell movement within a sheet. Our findings suggest that APC-driven actin nucleation promotes cell junction integrity and dynamics to facilitate collective cell remodeling and consequent motility. Our study offers a new perspective to explore the relevance of APC-driven cytoskeletal function in gut morphogenesis.

## Póster 7

Diego Belda(1, 2); Luca de Vincenti ( 2); María Sanz (3); Belén Castro (2) and Montse Bosch (2).

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### **Evaluation of enzyme-based and natural extracts-based products for biofilm control.**

A biofilm is an aggregate of microorganisms in which cells are embedded in a self-produced extracellular matrix, facilitating cell-to-cell and cell-to-surface attachment. The biofilm matrix is composed of water and extracellular polymeric substances (predominantly consists of polysaccharides, proteins, and nucleic acids) and provides new advantageous functions for the bacteria. In the meat industry, biofilms formed by pathogenic and spoilage microorganisms have been identified. If biofilms are not completely eliminated, they can serve as a source of cross-contamination in foods, therefore compromising food quality and safety. Consequently, the development of new hygienic strategies is imperative to prevent and eliminate biofilms that act as reservoirs for these bacteria on the machinery and surfaces involved in meat processing. This project evaluates the antibiofilm capacity of E (a mixture of enzymes that disintegrate the matrix) and EE (the same mixture of enzymes supplemented with antimicrobial extracts with biocidal activity) products on a continuously generated *Pseudomonas aeruginosa* biofilm using a Drip Flow Reactor. The results indicate that product EE shows biocidal activity but neither product E nor EE shows disintegrant activity.

## Póster 8

Irene Boscá-Sánchez (1), Jesús Rodríguez-Díaz (1) y María J. Yebra (1)

(1) Instituto de agroquímica y tecnología de los alimentos (CSIC), Valencia.

### **Glicosil hidrolasas aisladas del metagenoma de la microbiota fecal de niños lactantes con actividad en N-glicanos.**

El N-glicoma asociado a las proteínas de la mucosa y de la leche humana constituye un conjunto de N-glicanos de gran complejidad estructural. Estos N-glicanos son sustratos para la microbiota gastrointestinal y también intervienen en muchas funciones biológicas, como la adhesión de patógenos, la señalización celular y la inmunomodulación. La N-glicosilación tiene lugar en una secuencia proteica consenso, en la que el disacárido N-acetilglucosamina(GlcNAc)- $\beta$ 1,4-GlcNAc (ChbNAc) está unido covalentemente a una Asn. La N-glicosilación se extiende con el complejo multianexo, que puede ser de alto contenido en manosa o tener estructuras de oligosacáridos híbridos y complejos que llevan además galactosa y GlcNAc. Estos últimos pueden estar a su vez fucosilados y/o sialilados.

La deglicosilación secuencial de los N-glicanos se lleva a cabo por glicosidasas específicas pertenecientes a diferentes familias de glicosil hidrolasas (GH). El objetivo de este trabajo es el análisis del metagenoma de la microbiota intestinal de niños lactantes con el fin de identificar glicosidasas implicadas en la hidrólisis de N-glicanos y estudiar su función en la interacción entre la microbiota, el huésped y virus entéricos. Utilizando el servidor dbCAN2 para la anotación funcional se han identificado 114  $\beta$ -galactosidasas (GH2), 35 exo- $\beta$ -N-acetilhexosaminidasas (GH20) y 16 endo- $\beta$ -N-acetilglucosaminidasas (GH18), que son familias para las cuales se ha descrito alguna glicosidasa con actividad en N-glicanos. Estas enzimas están ampliamente extendidas en los géneros *Bacteroides*, *Phocaeicola*, *Bifidobacterium* y *Ruminococcus* y también se encuentran presentes en especies de los géneros *Megasphaera*, *Klebsiella*, *Streptococcus*, *Actinomyces*, *Enterococcus*, *Parabacteroides*, y *Limosilactobacillus*.

Basándose en homología de secuencia, se han seleccionado 3 genes que codifican para endo- $\beta$ -N-acetilglucosaminidasas GH18 (Endo11, Endo38 y Endo358) para ser clonados y caracterizados. Las tres enzimas han mostrado diferente especificidad de substrato. La Endo38 fue la única con actividad frente a glicoproteínas, siendo capaz de hidrolizar el enlace  $\beta$ 1,4 del disacárido ChbNAc de los N-glicanos de alto contenido en manosa, híbridos y complejos, siempre y cuando estos últimos no estén fucosilados ni sialilados. También es capaz de hidrolizar oligosacáridos libres como el dímero y el trímero de GlcNAc. La enzima Endo11 presenta actividad hidrolítica frente a oligosacáridos compuestos por dos o tres moléculas de GlcNAc, mientras que la Endo358 muestra actividad endo-quitinasa en el tetrámero de GlcNAc. Se han clonado también dos genes que codifican para  $\beta$ -galactosidasas GH2 (Gal1b y Gal37c). Ambas enzimas presentan actividad frente al substrato artificial p-nitrofenil- $\alpha$ -D-galactopiranósido. Gal1b es específica en la hidrólisis de enlaces  $\beta$ 1-4, liberando galactosa del extremo reductor del péptido G2, de los N-glicanos conjugados a la IgG, la lactoferrina bovina y la asialofetina, y de oligosacáridos libres. Estos resultados dan una idea de la gran diversidad de glicosidasas presentes en la microbiota gastrointestinal, apoyando la hipótesis de que los N-glicanos conjugados a proteínas son importantes para el establecimiento de la composición de la microbiota de los lactantes. Además, la degradación sucesiva de los N-glicanos influiría en el papel que desarrollan las glicoproteínas en el reconocimiento celular, y que no sólo se da entre las propias células sino también con organismos patógenos, por lo que las enzimas glicosidasas caracterizadas aquí poseen un gran potencial biotecnológico.

## Póster 9

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### Targeting MCL1-BOK TMD Interaction in Lung Cancer

The permeabilization of the outer mitochondrial membrane mediated by proapoptotic BCL-2 proteins is a critical event in apoptosis induction. The interaction between the different members of this protein family defines the cell fate. Our recent research describes the interaction between the anti-apoptotic myeloid leukemia cell differentiation protein (MCL1) and the pro-apoptotic BCL2-related ovarian killer (BOK) through their transmembrane domains. We have identified a novel small-molecule, MBolN 179, that disrupts this interaction, releasing BOK and promoting cell death.

Overexpression of MCL1 is frequently found in cancers and correlates with metastasis and resistance to conventional treatments. Particularly, in lung cancer MCL1 amplification is found in the 54% of tumors. Current MCL1 inhibitors target the cytosolic domain releasing the pro-apoptotic protein Bak. However, clinical trials have been halted due to cardiotoxicity. MBolN 179 offers a new possibility of inducing cell death in cells overexpressing MCL1 by releasing BOK that has a low expression in cardiomyocytes.

In this work, we have analyzed the expression of BOK and MCL1 in a panel of Lung cancer cell lines and measured the activity of MBolN 179 in 2D and 3D cultures.

Our findings emphasize the significance of the MCL1-BOK transmembrane domain interaction in lung cancer and highlight the potential of MBolN 179 as a therapeutic candidate.

## Póster 10

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### Caracterización de los fibroblastos asociados a tumores malignos (CAFs) que expresan fibronectina con el sitio sinérgico inactivado

Durante la progresión del cáncer se producen distintos cambios en la arquitectura de la matriz extracelular (MEC) y en su composición química: hay un aumento de MEC fibrosa por la acción conjunta de metaloproteinasas, Lisil-oxidases y los fibroblastos asociados al cáncer (CAFs) que están muy activados, es decir son miofibroblastos con mayor actividad contráctil debido a la expresión de  $\alpha$ -actina de músculo liso ( $\alpha$ SMA), lo cual provoca la reorientación de las moléculas de colágeno generando fibras más rígidas. Si la MEC que

rodea las células es rígida, las fuerzas mecánicas generadas sobre las células se transmiten a través de integrinas e inducen cambios en el citoesqueleto. A su vez, estos cambios estimulan vías señalizadoras que activan respuestas celulares como incrementos en la proliferación y migración.

La glicoproteína fibronectina (FN) es un componente mayoritario de las MECs que contiene motivos de unión a receptores celulares, como integrinas y sindecanos. El principal motivo de unión a integrinas en la FN es la secuencia RGD reconocida por  $\alpha 5\beta 1$ ,  $\alpha IIb\beta 3$  (solo en plaquetas) e integrinas que contienen av. De las integrinas que se unen al motivo RGD de FN,  $\alpha 5\beta 1$  tiene un importante potencial mecanotransductor. Esto se produce gracias a una segunda adhesión a una secuencia (DRVPPSRN) en el módulo adyacente de la FN, denominada sitio sinérgico, que refuerza esta adhesión de  $\alpha 5\beta 1$  con FN. Nuestro equipo generó una cepa de ratones que expresan FN con el sitio sinérgico inactivado por la mutación de sus argininas en alaninas, reduciendo así grado de conexión de las  $\alpha 5\beta 1$  con la FN. Esto nos permite evaluar la importancia de las adhesiones  $\alpha 5\beta 1$ -FN en el comportamiento de los CAFs de tumores de glándula mamaria procedentes de ratones portadores del transgén MMTV-PyVT. De acuerdo a nuestros resultados, los CAFs que expresan FN con el sitio sinérgico inactivado forman redes de FN menos densas y elaboradas, lo que lleva a la formación de MECs menos rígidas y menos fibrosas.

## Póster 11

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### Differences in virulence of *M. tuberculosis* ecotypes based on host specificity

Tuberculosis is one of the leading causes of death from infectious agents worldwide. It is caused by the *Mycobacterium tuberculosis* complex (MTBC) which is composed of 13 lineages or ecotypes. MTBC lineages can be broadly divided into animal and human-associated lineages. MTBC ecotypes possess a genomic similarity higher than 99%. However, the ecotypes differ among them in the host range they can infect. Knowing the host-pathogen interactions and their compatibility will allow us to decipher the factors that influence *M. tuberculosis* virulence.

We hypothesize that virulence is influenced by host compatibility and that *M. tuberculosis* strains show higher virulence phenotypes when infecting their favored host. To test this, we have used an in vitro infection system to explore the virulence between different hosts by infecting human and bovine macrophages with two strains of *M. tuberculosis* from human-associated lineages (Lineages L5 and L6) and two strains associated with animals *Mycobacterium bovis* (Lineage A1) and Chimpanzee bacillus (Lineage A4).

Our analysis revealed that the infection ratio is increased when cells are infected with their favorite strain. Additionally, these preferred host-lineage combinations resulted in decreased

cell viability and higher necrosis levels, in contrast to bacterial-host combinations that were not preferred. Finally, our preliminary results suggest that one of the necrosis mechanisms involved in *M. tuberculosis* infection is ferroptosis.

## Póster 12

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### **Characterization of the insertion in membranes of the pH-sensitive peptide LAH4 by perfusion-induced ATR-FTIR spectroscopy**

The spontaneous insertion mechanism of helical peptides in membranes is not fully understood. One problem is that transmembrane segments are hard to work with. One alternative is the use of pH-sensitive peptides such as LAH4, a histidine-rich alpha-helical peptide that changes its protonation state depending on the pH. Then, fast pH changes can be used to control its membrane topology. This requires first a reliable estimation of its apparent pKa for the insertion.

It has been reported that LAH4 adopts a transmembrane helix at high pH values but shifts to an interfacial helix as the His residues protonate, with an apparent pKa of 6 [1]. So far, this information has been obtained by using oriented membranes, either dry or partially hydrated, using solid-state NMR [1] or polarized ATR-FTIR spectroscopies [2], with several samples prepared at different pHs.

In this work we implemented a perfusion setup for polarized ATR-FTIR spectroscopy to follow the LAH4 topology in fully hydrated oriented membranes as the pH in contact with the sample was changed. This allowed us to obtain a pH titration curve from a single film, instead of preparing multiple samples at different pHs as previously done.

The linear dichroism FTIR spectra obtained at different pH conditions indicated that LAH4 inserts in membranes with an insertion pKa of 6.5. Further analysis will allow us to study the structural changes in a more dynamic system.

## Póster 13

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### **Understanding the role of the hexameric conformation in the bacterial transcriptional factor RcsB**

The transcriptional factor RcsB is a response regulator protein conserved in the Enterobacteriaceae family. It forms part of the Rcs system which senses cell envelope stress to achieve adaptation and survival that comprises the outer membrane sensor protein RcsF coupled to the inner membrane sensor protein IgaA, which is also coupled with the inner membrane phosphorelay proteins RcsD and RcsC1. Upon the activation of the phosphorelay proteins, RcsB becomes phosphorylated acquiring the activated conformation to regulate gene transcription affecting different functions such as repression of motility and production of an extracellular capsule. However, non-phosphorylated RcsB also shows gene transcription regulation. In the last years, thanks to our previous structural work we were able to obtain a dimerization conformation stabilized by phosphorylation that was able to bind a specific DNA box in a base readout mode<sup>2,3</sup>. However, we could also trap an alternative dimeric conformation of RcsB, that formed hexamers just in the crystals, pointing to a possible conformational dynamism involved in function<sup>2</sup>. Now, we have produced a mutational analysis in RcsB that has allowed us to stabilize the hexameric conformation, according to SEC-MALS analysis, which seems to bind DNA in an alternative mode taking into account base and shape readout.

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#### **Póster 14**

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#### **Study of the regulation of mitosis by the polo-like kinase Cdc5 in *Saccharomyces cerevisiae*.**

Exit from mitosis and completion of cytokinesis requires the inactivation of mitotic cyclin-dependent kinase (Cdk) activity. For this purpose, *Saccharomyces cerevisiae* needs to activate the mitotic phosphatase Cdc14 which is released from the nucleolus through the phosphorylation of its inhibitor Net1. Two pathways contribute to Cdc14 regulation: FEAR (Cdc14 early anaphase release) and MEN (mitotic exit network). Moreover, Cdc5 polo-like kinase was found to be an important mitotic exit component. However, its specific role in

mitotic exit regulation and its involvement in Cdc14 release remain unclear. For this reason, we want to clarify the mechanism by which Cdc5 contributes to the timely release of Cdc14. Our previous genetic and biochemical data indicate that Cdc5 acts in parallel with MEN during anaphase. This MEN-independent Cdc5 function requires active separase and activation by Cdk1-dependent phosphorylation. We also demonstrate that Cdc5 can induce the phosphorylation of Net1 in different residues to those phosphorylated directly by Cdk1. We identified the Net1 phosphorylation residues by mass spectrometry and are currently studying their relevance *in vivo* during anaphase. Analyzing the Net1 phospho-mutants we expect to gain new insights on the alternative activation route of Cdc14 independent of Cdk1.

## Póster 15

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## Computational modeling of DYRK1A inhibitors as potential anti-Alzheimer Agents

Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) is a promising target for the treatment of different neurodegenerative diseases, and especially Alzheimer's disease (AD). In this chapter, different ligand-based and structure-based computational approaches were explored to develop a workflow for the selection of potential candidates as inhibitors of DYRK1A. The NuBBe database -comprising different compounds from the Brazilian biodiversity landscape- was screened with the designed workflow allowing to search for potential inhibitors. Five different compounds from this database were identified as candidates, and one of them not only presented a good interaction profile with the ATP binding site of DYRK1A, but also a great synthesizable accessibility score and an optimal predicted toxicological profile. These results show the capability of the developed *in silico* workflow to screen large databases to find hit compounds from natural sources, therefore representing a good starting point for further future studies.

## Póster 16

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## Rationalizing the chemical evolution of Antimonene in biological conditions with its biomedical performance.

Nanomedicine has emerged as a promising field for improving disease diagnosis and treatment by harnessing the unique properties of nanosized or nanostructured materials. Antimonene (AM), a two-dimensional (2D) allotrope of antimony with a distinctive buckled honeycomb structure, emerges as a fascinating nanomaterial for various biomedical applications. AM exhibit remarkable properties for nanomedicine, including a tunable bandgap and excellent photothermal conversion efficiency, as well as the on-demand generation of reactive oxygen species (ROS).[1, 2] These properties make AM an appealing candidate for various biomedical applications, such as biosensing, photothermal cancer therapy, and theragnostics.

Nevertheless, while the field of nanomedicine has witnessed remarkable advancements, the clinical application of nanomaterials remains limited. To overcome this hurdle, future research in nanomedicine must focus on developing new materials with improved properties, exploring alternative and scalable fabrication processes, understanding the interactions between nanomaterials and biological systems, and standardizing toxicity evaluation protocols. In this regard, the practical application of AM also faces challenges related to morphology homogeneity, surface oxidation, particle size distribution, and scalability. Additionally, the stability of bare AM in biological conditions remains understudied, as most investigations have focused on coated AM-based materials.

To address these challenges, herein we present the first study on the stability of AM nanomaterials in cell culture media. Using synchrotron-based *in situ* X-ray absorption spectroscopy (XAS), the chemical evolution of three different bare AM-based nanomaterials under biological conditions have been explored, shedding light on its degradation. Furthermore, we correlate the chemical evolution of AM with the toxic effects it produces in 6 different cell lines, shedding light on the mechanisms behind these effects. We believe the systematic study here presented will pave the way for the rational design and functionalization of AM, to enhance its biomedical performance in medical treatments.

### Póster 17

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## Regulación de la síntesis de polifosfato en *Lacticaseibacillus paracasei*

En bacterias lácticas (BAL) la producción del polímero lineal de fosfato (Pi) polifosfato (poli-P), juega un papel importante a nivel probiótico, participando en el mantenimiento de la homeostasis intestinal. En *Lacticaseibacillus paracasei* BL23 se han identificado los genes *pstSCAB1B2-phoU*, que codifican un posible transportador ABC de alta afinidad para el Pi (*pstSCAB1B2*) y un regulador de su actividad (*phoU*), y los genes *phoPQ*, que codifican un

sistema de regulación de dos componentes que controlaría la expresión de genes relacionados con el Pi. En otros organismos, se ha descrito que la enzima polifosfato quinasa (Ppk) es la responsable de la síntesis de poli-P, mientras que su degradación es llevada a cabo por la enzima exopolifosfatasa (Ppx). En *L. paracasei* BL23, el gen que codifica Ppk está agrupado con dos genes que codifican exopolifosfatasas con diferente composición de dominios, con el orden génico ppx1-ppk-ppx2, pero la función de estos genes ppx nunca se ha explorado.

*L. paracasei* BL23 sintetiza y acumula poli-P en forma de gránulos intracelulares de manera transitoria, coincidiendo con la entrada en la fase estacionaria. Se construyeron mutantes en los genes ppx1 y ppx2 en esta cepa. Sorprendentemente, y al igual que una mutación en ppk, la eliminación del gen ppx1 dio como resultado la incapacidad de acumular poli-P, mientras que la mutación de ppx2 no tuvo ningún efecto en la síntesis. La expresión de ppk no varió en el mutante ppx1 y la transformación del mutante ppx1 con un plásmido que expresa el gen ppk constitutivamente no restauró la producción de poli-P. En cambio, la restauración de la síntesis de poli-P en esta cepa se obtuvo después de la expresión de ppx1 en trans, excluyendo así efectos polares en la expresión de ppk como la causa de la ausencia de poli-P. Estos resultados demuestran que Ppk y Ppx1 son ambas necesarias para la síntesis de poli-P en *L. paracasei*. Adicionalmente, se comprobó el efecto de otros genes del metabolismo del Pi sobre los niveles de poli-P mediante la construcción de cepas deficientes. Cepas con mutaciones en phoP (regulador de la respuesta) y pstC (componente del transportador ABC de Pi) fueron incapaces de acumular poli-P. Mientras que tras la inactivación de phoQ (quinasa sensora), la síntesis se redujo a la mitad aproximadamente. Sin embargo, un mutante en phoU produjo cantidades de poli-P por encima de los de la cepa silvestre.

Nuestros resultados muestran que la producción de poli-P en *L. paracasei* está estrictamente regulada. Además, estos resultados revelan un escenario inesperado y complejo para la contribución de las exopolifosfatasas a la síntesis de poli-P.

### Palabras claves:

*Lacticaseibacillus paracasei*, polifosfato, polifosfato quinasa, exopolifosfatasas, transportador de fosfato, sistema de dos competentes.

### Póster 18

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## Repurposing the mammalian RNA-binding protein Musashi-1 as an allosteric translation repressor in bacteria

Gene regulation at the post-transcriptional level is pervasive in living organisms of ranging complexity. Indeed, the ability to regulate at different points of the genetic information flow appears instrumental to maximize the integration of intrinsic and extrinsic signals, which enables an efficient information processing by the organisms. However, the solutions implemented in prokaryotes and eukaryotes greatly differ. In prokaryotes, for instance, a series of RNA-binding proteins support translation initiation. By contrast, eukaryotes deploy a sizeable number of RNA-binding proteins with a variety of functions. In animals, in particular, most RNA-binding proteins contain RNA recognition motifs (RRMs). RRMs are highly plastic domains of about 90 amino acids that fold into four antiparallel b-strands and two a-helices and can bind to single-stranded RNAs with enough affinity and specificity. Yet, the preponderance of RRMs does not occur in all organisms. In fact, the scarcity of RRM-containing proteins in prokaryotes and the partly unknown functional role of those identified by bioinformatic methods question if RRMs can readily work in these organisms with much simpler gene expression machinery and intracellular organization. To address this intriguing question, we adopted a synthetic biology approach. In this work, we used the mammalian RNA-binding protein Musashi-1 (MSI-1) as a translation repressor in the bacterium *Escherichia coli*. MSI-1 contains two RRMs in the N-terminal region and recognizes the RNA consensus sequence RUnAGU on the nanomolar scale. In addition, MSI-1 can be allosterically inhibited by a fatty acid. In mammals, MSI-1 regulates Notch signaling by repressing the translation of a key protein in the pathway. Hence, rather than moving genetic elements from simple to complex organisms, as it is normally done, we reversed the path by moving an important mammalian gene (from *Mus musculus*) to *E. coli*. Some eukaryotic factors have already been implemented in bacteria to regulate gene expression at different levels, but the case of RRM-containing proteins remains elusive. In the following, we present quantitative experimental and theoretical results on the response dynamics of a synthetic gene circuit in which MSI-1 is regulated transcriptionally by the lactose repressor protein (LacI), and it works as an allosteric translation repressor by means of a specific interaction with an mRNA (encoding a reporter protein) that harbors a suitable binding motif.

### Póster 19

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## Deciphering the role of Protein Kinase C (PCK) in the DNA integrity checkpoint: from yeast to mammals and back

In response to DNA damage, cells activate the DNA integrity checkpoint, a mechanism responsible for initiating DNA repair and halting cell division until the damage is repaired.

Our research group has identified Protein Kinase C (PKC) as a key player necessary for the activation of this checkpoint1, which we detect as phosphorylation of the effector kinase Rad53 in yeast or CHK1/CHK2 in mammals. Here we present our strategy to establish the role of PKC in the DNA integrity checkpoint using different model organisms. We use *S. cerevisiae* to study the molecular mechanism of checkpoint control. Pkc1 is necessary for the recruitment of Dpb11 and Rad9, phosphorylation of Ddc1, Rad9 and Rad53 as well as the interaction of Rad53 and its scaffold Rad9. Our results suggest that the activity of the sensor kinase Mec1 may be affected in a *pkc1*- mutant, which we aim to determine in future experiments. In mammalian cells, PKC $\delta$  is known to contribute to the activation of the DNA integrity checkpoint1. We found that the checkpoint is only partially affected in PKC $\delta$ -/- mutant lines of mouse embryonic stem cells (ESCs) and neural stem cells (NSCs). To study whether other PKC isoforms could be also involved, we performed a functional screening in yeast and found that PKC $\theta$  is also capable to activate the checkpoint. We are currently generating PKC $\delta$  and PKC $\theta$  knock-out NSC lines to assess the contribution of the latter to the regulation of the DNA damage checkpoint. In the future, we plan to use this mammalian model to study if the molecular mechanism defined in yeast is also conserved. All together, our research will provide a better understanding of the role of PKC in the DNA integrity checkpoint.

## Póster 20

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## Identification of a novel ASC-dependent inflammasome inhibitor

Inflammasomes are a family of cytosolic multiprotein complexes that assemble in response to pro-inflammatory stimuli. The activation of different inflammasome receptors converge in the formation of ASC oligomers (Apoptosis-associated Speck-like protein containing a Caspase recruitment domain), which recruit and activate procaspase 1 (PC1). Active caspase 1 triggers the maturation of pro-inflammatory cytokines like interleukin (IL)-1 $\beta$ , IL-18 and cleaves Gasdermin D, resulting in the spread of the inflammatory signal and in the induction cell death through pyroptosis. While inflammasomes play a critical role in innate immunity, their overactivation has been associated with several pathologies, including Alzheimer's disease, type II diabetes, cancer, and inflammatory diseases. Activation of different inflammasomes has been demonstrated in these diseases. Consequently, the identification of pan-inflammasome inhibitors is crucial for mitigating the impact of over-inflammation on these multifactorial diseases.

In this study, we present a new molecule called QM372, which demonstrates the ability to prevent ASC-mediated activation of procaspase-1 *in vitro*. Treatment with QM372 also exhibits a reduction in the processing of IL-1 $\beta$  and attenuates pyroptosis in both cell lines and primary cell cultures. Taken together, these findings introduce QM372 as a promising broad-spectrum inflammasome inhibitor that holds potential as a therapeutic agent for complex inflammation-associated diseases.

## Póster 21

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## Exploring the Transmembrane Domains of the Bcl2 Family: A Comprehensive Study

Apoptosis is the main mechanism of programmed cell death. There are two pathways that lead to the activation of apoptosis, the intrinsic and the extrinsic pathways. The intrinsic apoptosis pathway is activated in response to intracellular danger signals and regulated by the B-cell lymphoma 2 (Bcl2) protein family. According to their function the Bcl2 family can be divided into two functional categories: pro-apoptotic proteins, which promote cell death, and anti-apoptotic proteins, which prevent apoptosis. All members of the Bcl2 family share some sequence homology domains. Furthermore, most members of the Bcl2 family possess a transmembrane domain (TMD) at the carboxyl-terminal (Ct) end, allowing their insertion into the lipid bilayer. We have demonstrated that these TMDs participate in protein-protein interactions within the Bcl2 family and, more importantly, in the control of apoptosis. Our research focuses on characterizing the intricate network of TMD interactions within the Bcl2 family using various approaches. On one hand, we experimentally determine the interacting partners and study the strength of their interactions. Our investigations encompass not only one-to-one interactions but also more complex interactions that reflect the presence of multiple transmembrane domains simultaneously, bringing us closer to understanding the cellular context. On the other hand, we aim to understand the structural basis of these TMD-TMD interactions, exploring the possibility of a multi-interaction model or a key-lock model.

## Póster 22

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## Establishment of patient-derived organoids to assess drug responses based on specific tumour features in colon cancer.

Colorectal cancer is a heterogeneous group of neoplastic diseases affecting 1.9 million people annually. Each patient presents a distinct genetic background, varying the response to treatment. Moreover, the tumour location in the right or left colon seems to be an important factor in response to therapy. In this context, patient-derived organoid (PDO) models can help predict patient response and study the molecular mechanisms underlying the response to treatment moving forward to personalised medicine.

In this project, two tumoral and normal organoid lines derived from colon cancer tissue have been efficiently established and followed-up. Normal PDOs present a better outcome than their tumoral PDOs counterpart. In addition, drug sensitivity assays have been performed on two PDOs derived from right-sided and left-sided tumours of the colon. When treated with chemotherapy drugs, a higher effect of 5-fluorouracil, oxaliplatin and their combination was seen in the left-sided organoid line, as expected, which makes organoids valuable models to study the molecular mechanisms underlying this differential response in patients. On the other hand, when combined with SN-38, a higher effect was seen on the right-sided organoid line, while treatment with SN-38 alone seemed beneficial independently on the tumour location. Furthermore, when targeted therapies were administered to PDOs according to their mutational profile, a higher effect of alpelisib (a PI3K inhibitor) was observed on the PI3K mutated organoid line. In addition, the unexpected results obtained for the treatment with two more drugs targeting other genes highlight the role of tumour heterogeneity in drug response and, therefore, the relevance of PDOs as personalised models.

## Póster 23

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## Functional Approaches for Inhibition of Dimeric Bacterial Transcriptional Factors Involved in Virulence

RcsB is the regulator of the response of the phosphorelay Rcs system, which is activated in response to stresses on the outer membrane, such as treatment with polypeptide antibiotics such as polymyxin B (1). RcsB is conserved in the Enterobacteriaceae family and acts as a transcriptional factor controlling a large number of genes related to the biosynthesis of the capsule, the flagellum, resistance to antibiotics, the formation of biofilms or virulence, among others. RcsB belongs to the NarL/FixJ response regulator family and its activation causes its phosphorylation and homodimerization to regulate the transcription of hundreds of genes (2, 3). In order to study the inhibition of the RcsB dimerization process, a bimolecular fluorescence complementation (BiFC) approach has been developed. This is based on fusing two complementary non-fluorescent fragments of the Venus fluorescent protein to RcsB, so that the reconstitution of the two fragments produced by RcsB dimerization reconstitutes fluorescence (4).

For this, two constructs have been produced, one where the N-terminus of Venus was fused with RcsB in the vector pETNKL 1.1 and another where the C-terminus of Venus was fused with RcsB in the vector pETNKL 1.5. In vivo tests were also carried out with competent cells deleted in RcsB, which expressed said constructs by Western Blot detection with purified specific anti-RcsB antibodies. Likewise, motility and encapsulation assays were carried out, which made it possible to observe the effect of the expression of the constructions containing Venus fragments with respect to the strain that did not present RcsB expression or expressed the wild-type protein. On the other hand, activation of the Rcs system by treating cells with polymyxin B caused weak fluorescence, indicating that dimerization by RcsB phosphorylation did not lead to Venus reconstitution as expected. Finally, two inhibitory peptides were designed, based on the structural data of the RcsB dimer (3), which allowed their interference in both dimerization and DNA binding to be detected using native gels and EMSAs.

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### **Póster 24**

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### **Deciphering the role of fibronectin synergy site in mammary gland malignant tumors**

Fibronectin (FN) is an essential glycoprotein of the extracellular matrix (ECM) necessary during development, though it is also involved in tissue remodeling after injury or tumor progression and metastasis. FN binds integrins and triggers cell adhesion, several signalling pathways and cytoskeleton organization regulating cell migration and proliferation. The most important cell binding site in FN is a RGD motif, whose engagement by some integrins ( $\alpha 5\beta 1$  and  $\alpha 1\beta 1\beta 3$ ) is reinforced by the union of the so called synergy site motif in the adjacent module to the one containing the RGD. FN is accumulated in the tumor stroma, secreted and assembled into fibrillar matrices by cell types such as Cancer Associated

Fibroblasts (CAFs) and Tumor Associated Macrophages (TAMs) creating the ideal microenvironment to favour cancer development and tumor cell migration. In our laboratory we are studying the role of fibronectin synergy site in mammary gland tumors to understand its involvement in cell adhesion and tumor progression, and its possible use as a target to treat breast cancer.

Our preliminary results demonstrate that inactivating the fibronectin synergy site *in vivo* (FnSyn/Syn mice) causes a delay in the tumor onset and tumor growth rate in mice carrying the MMTV-PyVM transgene that induces the apparition of malignant tumors the mammary glands. We analysed the *in situ* proteomic profile of the tumors and observed a significant reduction in genes involved in mRNA translation in FnSyn/Syn mice compared to mice expressing wild type FN. However, it seems that cell proliferation is not affected by this mutation. Proteomic analysis of stromal regions of the tumors shows a reduction in ECM proteins, integrins and other proteins related to them in the FnSyn/Synmice compared to the wt ones. Our data suggest that the inactivation of fibronectin synergy site leads to a less fibrotic stromal environment in the tumor.

## Póster 25

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### A photoswitchable helical peptide with light-controllable interface/transmembrane topology in lipidic membranes

The spontaneous insertion of hydrophobic helical peptides into lipid bilayers is a process not fully understood [1]. This is in part due to the lack of sensitive experimental procedures allowing to perturb transmembrane peptides (TM) out of equilibrium reversibly and in a small-time frame and measure with sufficient spatial and temporal resolution the changes in their topology and structure. A practical approach to achieve this sensitivity is by photocontrol, using for instance a molecular photoswitch coupled to the peptide, such as an azobenzene derivative. The fast and reversible photoisomerization of the azobenzene group from trans to cis conformation can induce changes in the structure and polarity of the peptide and, hence, promote a transmembrane-to-interphase (TM-to-MI) transition as we have shown recently [2].

In this work, we introduced a hydrophobic helical peptide covalently coupled to an azobenzene photoswitch (KCALPazo), which shows a light controllable TM/MI equilibrium in hydrated lipid bilayer POPC films accordingly to orientation measurements from FTIR spectroscopy [2]. However, we also concluded that only a fraction of the peptide moves to the interface upon azobenzene photoisomerization to cis. We have now looked for variations of KCALPazo, with the goal to obtain a photoswitchable peptide whose membrane topology shows an enhanced response to azobenzene photoisomerization. Our results might help to

design photoswitchable hydrophobic peptides suitable to control and study the insertion of helices in membranes.

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## Póster 26

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### Evaluación de los niveles de antioxidantes en la diabetes tipo 1 e impacto del entrenamiento HIIT y MICT

#### Introducción:

La diabetes tipo 1 (DM1) es una enfermedad crónica cuyas alteraciones metabólicas llevan a un desequilibrio en el estado redox. La actividad física se presenta como una intervención para mejorar el estado redox. Las dos metodologías más utilizadas son el entrenamiento extensivo de intensidad moderada (MICT, 50-75% VO<sub>2</sub>máx) y el ejercicio interválico de alta intensidad (HIIT, 85-150% VO<sub>2</sub>máx). Se desconoce si el ejercicio aeróbico tipo MICT o HIIT podría tener un efecto antioxidante en pacientes con DM1.

#### Objetivos:

- 1) Evaluar si la expresión de enzimas antioxidantes está disminuida en sangre de pacientes con DM1, en comparación con sujetos sanos.
- 2) Analizar los efectos de dos metodologías distintas de ejercicio físico sobre la expresión de enzimas antioxidantes en pacientes con DM1.

#### Material y métodos:

Se realizó una extracción de sangre a 26 pacientes con DM1 y 25 sujetos control. Posteriormente de los 26 pacientes con DM1, 8 realizaron ejercicio MICT (30 minutos continuos a una intensidad del 65-70% del VO<sub>2</sub>máx) y 18 realizaron HIIT (10 repeticiones de 30 segundos al 100-120% del VO<sub>2</sub>máx y 2 minutos de descanso activo al 40-50% del VO<sub>2</sub>máx). Ambos grupos realizaron un calentamiento previo y una vuelta a la calma al finalizar el entreno de 5 minutos, entrenaron 3 días a la semana durante 3 semanas (9 sesiones en total). Se extrajo el RNA total de sangre, siguiendo el protocolo de NucleoSpin® RNA blood kit (Marchery-Nagel) para posteriormente analizar la expresión de

los enzimas antioxidantes: Glutation peroxidasa 1 (GPX1), catalasa (CAT), superóxido dismutasa (SOD) y glutatión-disulfuro reductasa (GSR); así como de genes reguladores del estado redox: gen codificante del factor nuclear eritroide 2 (NFE2L2) y Sirtuina 1 y 3 (Sirt1/3) mediante la técnica de RT-qPCR. Se normalizó la expresión génica utilizando el gen 18S y se calculó la expresión relativa mediante el método 2- $\Delta\Delta CT$ .

### **Resultados:**

Respecto al grupo control, los pacientes con DM1 presentaron una menor expresión de los genes antioxidantes GPX1 (diferencia entre medias [unidades arbitrarias] =  $-60,6 \pm 17,3$ ; p-valor < 0,01) y CAT (diferencia entre medias =  $-45,3 \pm 5,4$ ; p-valor < 0,001), así como de los genes reguladores NFE2L2 (diferencia de medias =  $-57,7 \pm 25,2$ ; p-valor < 0,05), Sirt1 (diferencia entre medias =  $-57,1 \pm 15,0$ ; p-valor < 0,01) y Sirt3 (diferencia entre medias =  $-31,3 \pm 13,9$ ; p-valor < 0,05). En cuanto al ejercicio físico en pacientes con DM1, no se encontraron diferencias significativas entre las medidas pre- y post-ejercicio para ninguno de los protocolos (HIIT y MICT). GPX1(HIIT, p-valor = 0,59 / MICT, p-valor = 0,75), CAT (HIIT, p-valor = 0,56 / MICT, p-valor = 0,91), SOD (HIIT, p-valor = 0,88 / MICT, p-valor = 0,28), GSR (HIIT, p-valor = 0,50 MICT, p-valor = 0,37), Sirt3 (HIIT, p-valor = 0,41 / MICT, p-valor = 0,44). Sin embargo, se observó una tendencia de reducción de la expresión de NFE2L2 en el protocolo HIIT (p-valor = 0,08), mientras que para el MICT no hubo cambios significativos (p-valor = 0,52).

### **Conclusión:**

Nuestros resultados muestran que en pacientes con DM1 hay una disminución de enzimas antioxidantes en sangre y que ninguno de los protocolos de intervención con ejercicio físico, MICT o HIIT, ha tenido el suficiente impacto sobre los sistemas antioxidantes como para revertir este descenso.

### **Agradecimientos:**

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## **Póster 27**

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**Papel de LIGHT/TNFSF14 en las poblaciones linfocitarias en el contexto de la arteriosclerosis.**

**Introducción y objetivos:**

Estudios previos señalan una participación de LIGHT(TNFSF14) en la arteriosclerosis. En el modelo murino deficiente para la apolipoproteínaE (Apoe-/-), la deficiencia de LIGHT agrava el ateroma alterando los órganos linfoides terciarios arteriales, conduciendo también a un aumento en las poblaciones linfocitarias circulantes de CD4+CXCR3+Th1/CD4+CCR4+CCR6+Th17 y un descenso de CD4+CD25+Foxp3+Treg. En esta investigación, se estudió en mayor profundidad el papel de LIGHT a través de estudios transcriptómicos *in vivo* de ateroma de ratones Apoe-/- y Apoe-/-Light-/- y estudios *in vitro* utilizando linfocitos humanos.

**Métodos:**

Los ratones Apoe-/- y Apoe-/-Light-/- fueron alimentados con dieta aterogénica durante 12 semanas. Los ratones fueron sacrificados para su caracterización histopatológica en cortes trasversales aórticos y para su análisis de expresión génica por secuenciación de mRNA (RNAseq) del cayado aórtico libre de adventicia. Para estudiar el papel de LIGHT *in vitro*, linfocitos CD4+ aislados de sangre humana fueron cultivados, diferenciados (durante 7 días) y tratados con LIGHT durante 72 horas para su análisis por citometría de flujo y RT-qPCR. El efecto de LIGHT en la proliferación de células T fue estudiado a través del análisis de la incorporación de BrdU durante 24 horas en los linfocitos tratados con LIGHT.

**Resultados:**

El estudio de RNAseq mostró que los ratones Apoe-/-Light-/- infraexpresaban diferentes genes relacionados con la diferenciación de linfocitos, su patogenicidad y prolifieración, como Cd5l, Tnfrsf1b, Cdkn1c y Syk. El tratamiento de los linfocitos CD4+ con LIGHT incrementó el porcentaje de T reguladores, el ratio Treg/Th17, la proliferación de los linfocitos protectores/antiinflamatorios Th2 y la expresión de Tec/Cd5l (reguladores negativos de la patogenicidad de Th17) y Cdkn1c (inhibidor de la proliferación celular).

**Conclusiones:**

Los resultados sugieren que LIGHT modula la arteriosclerosis a través de la regulación de las poblaciones linfocitarias, particularmente promoviendo fenotipos de células T protectores a través de la modulación de la proliferación.

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PI19/00169 y PI22/00062 (Instituto de Salud Carlos III), fondos FEDER, BIB-07-20 (SEA/FEA) y Proyecto Paula.

**Póster 28**

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### **Role of TET3-mediated demethylation in glioblastoma formation**

Glioblastoma multiforme (GBM) is the most common and aggressive primary tumour developed in the central nervous system. Genomic imprinting (GI) is an epigenetic phenomenon that causes the monallelic expression of imprinted genes in a parent-of-origin specific manner. Importantly, loss of imprinting (LOI) has been described as one of the most common events in human cancer. GI is mainly controlled by imprinting control regions (ICRs), that are differentially methylated regions (DMRs) in maternal and paternal chromosomes. Ten-eleven translocation (TET) enzymes are responsible for the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), playing an important role in imprinting regulation through demethylation. Working with a GBM cell line generated by the upregulation of the Egfr gene, we assessed the overexpression of TET3 in GBM cells (GBM-Tet3OE). We show that overexpression of TET3 enhances the capacity of GBM cells to give rise to bigger tumours when injected in the flank of immunocompromised mice. Moreover, mice intracranially injected with GBM-Tet3OE cells survived shorter periods than the ones injected with GBM control cells. Overexpression of GBM cells with TET3 containing a mutation of its catalytic domain (GBMCDmutTet3OE) results in a reduction in the size of the tumours developed by GBM cells in mice flanks, consistent with longer survival periods of intracranially injected mice. Notably, the analysis of expression of some imprinted genes in GBMTet3OE cells revealed significant alterations compared to the control condition. These alterations were not observed in GBMCDmutTet3OE cells, suggesting that TET3 might contribute to brain tumour growth in a demethylation-dependent way by regulating the expression of imprinted genes in GBM cells.

**Póster 29**

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### **Lipid composition of yeast cells modulates the response to iron deficiency**

Iron is an essential micronutrient that participates as a redox cofactor in multiple metabolic pathways, including lipid biosynthesis. In order to increase the intracellular bioavailability of iron when it is scarce, a group of genes known as the iron regulon is activated by the accumulation of the iron-responsive transcription factor Aft1 in the nucleus of the yeast *Saccharomyces cerevisiae*. However, we have observed that yeast cells with genetic defects in the biosynthesis of either unsaturated fatty acids (UFAs) or ergosterol display defects in iron regulon activation upon iron deficiency, which in turn contributes to limiting cell growth. Subcellular localization studies reveal that alteration of either UFAs or sterol levels lead to mislocalization of Aft1 protein to the vacuole under iron deprivation. The expression of an *AFT1* allele that is constitutively nuclear and activates the iron regulon regardless of the cellular iron status, *AFT1-1up*, partially rescues both the iron regulon activation defect and the growth defect displayed by cells with defective UFA or ergosterol biosynthesis under iron starvation. We are currently studying the molecular mechanisms responsible for Aft1 mislocalization to the vacuole when lipid biosynthesis is altered.

### Póster 30

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### **3D co-culture models of lung cancer tumorspheres and cancer associated fibroblasts: first approach to study their interactions.**

Cancer-associated fibroblasts (CAFs) are the most abundant stromal cells in the tumor microenvironment (TME) and play a critical role in the initiation, progression, and metastasis of lung cancer through their ability to enhance the stem properties of cancerous cells. Three-dimensional cell culture models, such as tumorspheres, are enriched of cancer cells with stem-like properties and offer a more accurate representation of the complex TME than other cell culture models. Despite the fact that TME has been widely studied, the molecular mediators involved in CAFs influence over cancer cells are still unknown.

**Póster 31**

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### **Role of TET2 in the epigenetic regulation of neural stem cells maintenance**

Neurogenesis in the mammalian brain is supported by multipotent neural stem cells (NSCs), which are characterized by their abilities of self-renewal and differentiation, giving rise to the three neural lineages: neurons, astrocytes, and oligodendrocytes. Several epigenetic processes have been implicated in neurodevelopment, neural plasticity, and differentiation. Genomic imprinting (GI) has been shown to be involved in the control of gene dosage in NSCs, regulating their maintenance and differentiation in the adult brain. Ten-eleven-translocation (TET) enzymes catalyze DNA demethylation, converting the 5-methylcytosine (5mC) into the epigenetic mark 5-hydroxymethylcytosine (5hmC) through an oxidation reaction. However, although hydroxymethylation levels are high in the mouse brain, the potential role of TET proteins in adult neurogenesis is not described. Here, we aim to understand the molecular mechanisms that control GI in adult NSCs, proposing the catalytic action of TETs as candidate modulator of imprinted genes expression by altering the methylation status of imprinting control regions (ICRs). To do this, first we have characterized the expression of Tet2 gene and its coding protein, TET2, both *in vivo* and *in vitro*, focusing on NSCs of the adult subventricular zone (SVZ) neurogenic niche. In addition, we have also studied the role of Tet2 in neurogenesis, by generating a conditional mouse model deficient in Tet2 specifically in NSCs. Our data reveal that TET2 is required for a proper differentiation of adult NSCs into non-neurogenic terminal astrocytes. Moreover, an RNA-Seq analysis in Tet2 deficient NSCs, has identified the TET2-regulated imprinted gene Thbs2 as a modulator of the differentiation process in NSCs. Finally, we show that Thbs2 is biallelically expressed in NSCs and that TET2 does not regulate the imprinted state of the gene, suggesting a methylation-independent role of TET2.

**Póster 32**

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### **Releasing BOK from transmembrane MCL-1 interaction: a novel strategy to induce cell death in tumors**

Induced myeloid leukemia cell differentiation protein (MCL-1) is an anti-apoptotic member of the B cell Lymphoma-2 (BCL2) family of proteins, overexpressed in more than 22 different tumours. Its overexpression is associated with poor prognosis and resistance to therapies.

MCL1 inhibitors, currently in clinical trials, target the cytosolic BCL2 homology 3 (BH3) domain and induce cell death mediated by apoptotic BAX/BAK executors. However, this therapy is not effective in several cases, such as those that decrease BAX expression or overexpress BCLxL. We have discovered that MCL-1 interacts with BCL2-related ovarian killer (BOK), another cell death effector member of the BCL2 family, through the transmembrane domain (TMD). We have identified a first-in-class drugs that breaks the transmembrane interaction between MCL-1 and BOK (MBolNs), releasing BOK to induce cell death. Our studies demonstrate that MBolNs treatment activates cell death by apoptosis in a BOK dependent and BAX/BAK independent manner. We have characterized MBolN activity as antitumor agent in different 2D and 3D tumour types. This novel mechanism of action would represent a new therapeutic alternative in oncology.

### Póster 33

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#### **Study of Start repressor Whi7 regulation by protein kinases**

The eukaryotic cell cycle covers sophisticated molecular mechanisms coordinated in space and time, whose final goal is the regulation of cell growth and division, according to the internal and external cellular signals. Start -also known as G1/S transition or restriction point in mammals- is the main decision point in which cells commit to a new round of cell division. Its molecular machinery is highly conserved in far-related organisms like mammals and yeast. In this work, we study the regulation by protein kinases of the Start transcriptional repressor Whi7 in *Saccharomyces cerevisiae*.

We first mapped *in vivo* Whi7 phosphorylation sites through phosphoproteomics. We identified twenty-one Whi7 phosphorylation sites, including nine of the twelve CDK (Cdc28/Pho85) predicted consensus sites. This assay also revealed unknown Whi7 phosphorylation sites, corresponding to RIM15 (nutrient depletion response), MEC1 (genome integrity checkpoint) and SLT2 (CWI pathway) among other protein kinases. We next focused on the multifunctional CDK Pho85, homolog of mammalian CDK5. We previously described that Pho85 phosphorylates Whi7 and downregulates its protein levels, through the repression of WHI7 expression and increasing Whi7 protein instability. Although Pho85 can regulate Whi7 protein levels in complex with multiple cyclins, here we identified Pcl1 as the main cyclin promoting Start activation through Whi7 inhibition. Besides, we analyzed the Pho85-dependent degradation of Whi7, and we found that Dma1 and Dma2 are the E3 ubiquitin ligase that target Whi7 for proteasomal degradation. Altogether, this work brings new insights about how Whi7 is regulated by the CDK Pho85 and proposes new protein kinases that could target Whi7. Future work will reveal whether and how these protein kinases regulate Whi7 function and under which cellular conditions.

**Póster 34**

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**Añadiendo piezas al puzzle incompleto de las enfermedades raras neurodegenerativas: nuevos genes y nuevas mutaciones.**

Las enfermedades raras (EERR) se definen en la Unión Europea como aquéllas que afectan a 1 persona de cada 2.000, y aunque una enfermedad rara afecta a muy pocos pacientes, el alto número de EERR que existen (más de 6.000) hace que haya un número elevado de afectados/as en todo el mundo (más de 300 millones). Se estima que el 72% de las EERR presentan una etiología genética, y el resto son el resultado de infecciones, alergias y causas ambientales. Por otra parte, el desarrollo de las nuevas tecnologías de secuenciación de nueva generación, como la secuenciación de exoma o de genoma, han permitido el descubrimiento de nuevos genes y nuevas mutaciones asociados/as a enfermedad, permitiendo el diagnóstico y favoreciendo el desarrollo de tratamientos para pacientes con EERR.

En el presente Trabajo Final de Máster se han analizado por filtrado y priorización de variantes de secuenciación de exomas, cuatro familias con fenotipo de ataxia o paraparesia espástica junto con otros signos clínicos. El objetivo es hallar la causa genética de la enfermedad mediante secuenciación de exoma (comprende la parte codificante del genoma). Los resultados han permitido resolver un caso índice con mutaciones en PI4KA, en dos probandos se identificaron variantes candidatas a ser mutaciones causales, y uno de los casos permanece sin diagnóstico.

Mutaciones en PI4KA causan un amplio espectro de patologías, desde retraso en el neurodesarrollo con hipomielinización y anomalías cerebrales del desarrollo a paraplejia espástica pura. El probando es heterocigoto compuesto para dos mutaciones novedosas: c.3845C>T y c.2750T>C. Predictores de patogenicidad determinaron que c.3845C>T afectaría al splicing, y mediante análisis de transcritos hemos concluido que genera un sitio críptico de splicing en el exón 33 que causaría la pérdida de 57 nucleótidos. Adicionalmente, hemos llevado a cabo un estudio de expresión para establecer la posible patogenicidad de una variante novedosa c.1120T>C detectada en un gen no asociado a enfermedad. Los hallazgos preliminares muestran que la proteína mutada tiene una expresión disminuida con respecto a la control.

**Póster 35**

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### **Estudio de los efectos del MitoGamide en las interacciones leucocito-endotelio de pacientes con diabetes tipo 2**

#### **Introducción:**

La diabetes tipo 2 (DM2) está relacionada con la disfunción mitocondrial y la alteración de las interacciones leucocito-endotelio. El metilgioxal es una especie de carbonilo reactivo subproducto de la glucólisis que aumenta de forma excesiva en la diabetes y reacciona con proteínas mitocondriales formando aductos de carbonilo, asociados a una mayor formación de especies reactivas del oxígeno. MitoGamide es un scavenger de metilgioxal con diana en la mitocondria y que podría modular las alteraciones mitocondriales y de interacción leucocito-endotelio.

#### **Objetivo:**

Determinar la biocompatibilidad de MitoGamide en modelos *in vitro* de neutrófilos de pacientes con DM2 mediante la evaluación de la viabilidad y la apoptosis, y su efecto en las interacciones leucocito-endotelio.

#### **Metodología:**

Para evaluar la citotoxicidad de MitoGamide se emplearon células de la línea celular humana leucocitaria HL60, que fueron incubadas durante 1h con diferentes concentraciones de MitoGamide (0-0,2-0,5-1-2,5-5 $\mu$ M). Se evaluó la viabilidad celular mediante un ensayo con CCK-8. La apoptosis se determinó mediante citometría de flujo y tinciones con Anexina-V y yoduro de propidio. Leucocitos polimorfonucleares (PMNs), aislados de muestras de sangre de pacientes con DM2 ( $n=11$ ) y sujetos control ( $n=12$ ), fueron incubados durante 1h con o sin MitoGamide. Pósteriormente, se analizó su interacción con células endoteliales (HUVEC) con y sin activación con TNF $\alpha$  en un sistema de adhesión dinámica *in vitro*.

#### **Resultados:**

La viabilidad celular y el proceso de apoptosis en células HL60 no se ven afectados a concentraciones de 0,1 a 5 $\mu$ M de MitoGamide. A partir de estos resultados y nuestra experiencia en compuestos con diana en la mitocondria se eligió la concentración de MitoGamide 0,5  $\mu$ M para continuar con los ensayos de interacción leucocito-endotelio. Los PMNs de pacientes con DM2 presentaron menor velocidad de rodamiento y mayor flujo de rodamiento y adhesión respecto a sujetos control. En los PMNs de los sujetos control, el tratamiento con MitoGamide incrementó la velocidad de rodamiento y redujo el flujo de

rodamiento y la adhesión de los leucocitos. En los PMNs de DM2, MitoGamide redujo significativamente la adhesión leucocitaria sin afectar la velocidad y el flujo de rodamiento. Al realizar este mismo ensayo de adhesión tras un tratamiento de 4h con TNFa, para inducir un estado pro-inflamatorio, observamos que tanto en sujetos control como en DM2, el MitoGamide es capaz de revertir las alteraciones en todos los parámetros de interacción leucocito-endotelio.

### **Conclusión:**

El compuesto MitoGamide no afecta a la viabilidad y proliferación celular ni induce apoptosis a concentraciones menores de 5 $\mu$ M. El tratamiento de PMNs con MitoGamide 0,5 $\mu$ M durante 1h reduce las interacciones leucocito-endotelio inducidas por inflamación, tanto en pacientes con DM2 como sujetos control indicando un papel protector para evitar el desarrollo de los procesos ateroscleróticos. Estos resultados sugieren el posible uso de esta molécula en la prevención de la atherosclerosis en pacientes con DM2.

### **Póster 36**

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### **Mathematical modelling of signalling pathways involved in chronological aging in *Saccharomyces cerevisiae***

The molecular mechanisms on which human aging is based have been extensively studied over the past decades, including metabolic and signalling pathways, and gene regulation. Furthermore, the mechanisms that induce or repress this phenomenon are also well known: cellular stress, nutritional restriction, radiation, etc. *Saccharomyces cerevisiae*, the budding yeast, has been widely used as a model organism to study, specifically, the signaling mechanisms involved in aging, being the most prominent ones the Ras/cAMP/PKA, TORC1/Sch9 and Snf1/AMPK pathways, due to the great homology between humans and yeast. In the present work, and based on the state of the art in the yeast signalling field, we develop a mathematical model based on ordinary differential equations (ODEs) implemented in Python and MatLab in order to predict cell behavior in its stationary phase under different nutritional regimens and radiation conditions. To calibrate the model, experimental data will be generated both in Valencia and in the Canfranc Underground Laboratory. The aim is contributing to elucidate the role of cosmic radiation in aging using an iterative method that combines *in silico* predictions with experiments to gain knowledge on the key molecular mechanisms involved (through the calibration, predictions and validation of the mathematical model). Then, we study the differential expression of genes at different times of aging using RNA-Seq experiments in order to see the evolution of gene expression in this phenomenon.

**Póster 37**

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### **Papel potencial del gen Guca1b en la aterosclerosis experimental**

#### **Antecedentes y objetivos:**

Recientes estudios de secuenciación de ARNm (RNA-seq) en nuestro laboratorio han demostrado una marcada regulación a la baja del gen Guca1b en el lecho vascular de ratones ateroscleróticos deficientes en apolipoproteína E (Apoe<sup>-/-</sup>) con desarrollo acelerado de la placa de ateroma asociado a la inactivación genética de la citocina LIGHT(TNFSF14). En este estudio, se exploró la posible participación de Guca1b en la progresión de la aterosclerosis y la modulación de su expresión por LIGHT en macrófagos estimulados.

#### **Métodos:**

La expresión de Guca1b se investigó mediante PCR cuantitativa (qPCR) en médula ósea (MO) y arco aórtico (AA) de ratones Apoe<sup>-/-</sup> sometidos a una dieta aterogénica durante 4, 8 y 12 semanas. Por otra parte, la expresión de Guca1b se analizó mediante qPCR en macrófagos derivados de la médula ósea (MDMO) de ratones Apoe<sup>-/-</sup> y Apoe<sup>-/-</sup>-Light<sup>-/-</sup> tratados con lipopolisacárido.

#### **Resultados:**

Como era de esperar, la caracterización histopatológica mostró que la lesión aumentaba con la dieta aterogénica, mientras que la expresión de Guca1b era tiempo y tejido-dependiente, con una marcada regulación a la baja a las 8 semanas, en comparación con las 4 y 12 semanas en AA y un aumento de expresión a las 8 y 12 semanas en comparación con las 4 semanas en MO. El análisis en MDMO reveló que Guca1b se expresa en macrófagos y que se regula a la baja en macrófagos proinflamatorios (estimulados con lipopolisacárido) caracterizados por un aumento de las citoquinas proinflamatorias IL6 y MCP1. Además, su expresión disminuye notablemente cuando se inactiva el gen Light, lo que sugiere que la expresión de Guca1b en los macrófagos depende de LIGHT.

#### **Conclusiones:**

La asociación inversa de la expresión de Guca1b con la progresión de la aterosclerosis y los macrófagos proinflamatorios sugiere un posible papel protector de Guca1b en la aterosclerosis mediante la modulación de la polarización de los macrófagos.

**Póster 38**

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### **Improvements in the genetic editing technologies: CRISPR-Cas and beyond.**

Gene editing is a great hope not only for the scientific community, but also for society in general. This is due to its potential therapeutic applications that would allow curing diseases of genetic origin. The first realistic approach to achieve this goal was the development of CRISPR tools. This review deals with some of the improvements that have been designed to obtain more efficient and safer genome editing. Initial CRISPR-Cas editing systems yield low efficiency and undesired editing products. To solve these problems, new approaches emerged, such as the creation of base editors. Recent discoveries have led to the development of many interesting alternatives, such as the CRISPR-associated transposable systems, which open the range by generating guided insertions, or the discovery of other programmable nucleases like the IscB family, which greatly increase the range of proteins available for editing uses. Also, to address the limitations of base editors, prime editors were created; this novel system, despite having some disadvantages compared to base editor systems, has the potential to generate all the possible point mutations. On the other hand, dual prime editing systems like twin prime editors, have been developed to create targeted insertions and enhance the editing outcomes, respectively. Furthermore, advances in gene editing do not reside solely in CRISPR-dependent systems, as we will discuss when treating the Replication Interrupted Template-Driven DNA Modification technique. Finally, bioinformatics is gaining a key role in the optimization of these systems, as well as the generation of new vector methods to direct these tools to their target cells.

**Póster 39**

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## Deciphering Mechanisms of Acquired Resistance to Sotorasib and Trametinib in KRAS- Mutated Non-Small Cell Lung Cancer

### **Introduction:**

Lung cancer is the deadliest type of cancer worldwide. Advances in research and the identification of molecular biomarkers, along with the development of targeted therapies, have allowed greater efficacy in patient treatment and improved prognosis. Approximately 20-25% of non-small cell lung cancer (NSCLC) cases have mutations in the KRAS gene, with KRAS p.G12C being the most common mutation. Recently, several specific inhibitors targeting this mutation or tyrosine kinases have shown promising results in patients with NSCLC. However, the development of acquired resistance is inevitable in targeted therapies.

### **Objective:**

This study aims to characterize the mechanisms that may be involved in the development of acquired resistance to tyrosine kinase inhibitors (TKIs) (Sotorasib and Trametinib) in KRAS-mutated NSCLC cells.

### **Methodology:**

A primary culture, PC435, established in the laboratory and mutated in KRAS G12C, as well as four KRAS-mutated NSCLC cell lines (NCI-H358: KRAS G12C, NCI-H23: KRAS G12C, A549: KRAS G12S, and NCI-H460: KRAS Q61H) were used. Resistance was generated by gradually increasing the concentration of Sotorasib in KRAS G12C-mutated cell lines and Trametinib in the rest cell lines evaluated. RNA seq was performed with the parental and resistant cell lines. Libraries were prepared using the ""Illumina TruSeq Stranded mRNA Prep"" kit, and RNA sequencing was performed using Illumina's NextSeq500 (Single-read, 75bp). Low-quality reads were removed using Trimmomatic, alignment to the human genome GRCh38 (Ensembl) was performed using STAR, and read counting was done with featureCounts. Resistant samples were compared with their corresponding parental cell lines, and differentially expressed genes (DEGs) were selected using EdgeR based on the following criteria: (i) Counts Per Million (CPM) greater than 1 in at least two samples; (ii)  $|\log_2\text{FoldChange}|$  greater than 1; and (iii) False Discovery Rate (FDR) less than 0,05.

### **Results:**

22 million reads per sample obtained from sequencing were ultimately converted to 17 million reads mapped to the reference genome, grouped into 62.710 genes. Principal component analysis reveals a clustering of the samples based on cell line and biological replicate. Furthermore, unsupervised hierarchical clustering of the expression of the 500 most variable genes demonstrates a clustering pattern according to cell differentiation phenotype (epithelial and mesenchymal). A gene set enrichment analysis (GSEA) was conducted using the ""Hallmarks"" from the MSigDB database. GSEA revealed alterations in genes: (i) upregulated by KRAS activation, (ii) involved in the epithelial-mesenchymal transition (EMT), (iii) related to the inflammatory response, and (iv) involved in cell cycle progression. Specifically, Sotorasib-resistant cell lines showed an overexpression of

SEMA6D and ABCA8, and downregulation of TMEM156, EREG, FOXA2, DUSP6, ITGA2, MYEOV, SPRY4, and ETV4. In Trametinib-resistant cell lines, no genes were found to be overexpressed, but a decreased expression of NT5E, DUSP6, UBASH3B, ETV4, and SPRY4 was observed.

### **Conclusions:**

The mechanisms of acquired resistance to Sotorasib and Trametinib in a patient primary culture and KRAS-mutated NSCLC cell lines studied are diverse and complex. Future therapeutic strategies involving rational drug combinations that inhibit multiple pathways and their clinical implications should be evaluated in further studies.

### **Acknowledgments:**

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### **Póster 40**

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### **SARS-CoV-2 Membrane Protein C-terminus tail plays a key role in virion assembly**

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Membrane (M) organizes the assembly and structure of new virions and is essential for virus formation (Yu et al., 2021; Finkel et al., 2021). It interacts with Envelope (E) protein (Kuo et al., 2016) to induce curvature in the membrane, thus allowing the assembly of viral particles and promoting maturation of Spike (S) (Boson et al., 2021). It also interacts with Nucleocapsid (N) protein to allow genomic RNA stabilization (Plescia et al., 2021). Recently reported cryo-EM structure of M protein confirms the presence of three transmembrane domains (TMDs) and a homo-dimeric conformation that might allow/promote viral assembly (Zhang et al., 2022; Dolan et al., 2022). In this work, we show that full-length M is retained in the ER until other structural proteins are present, then moving to the ERGIC-Golgi. On the contrary, expressing a truncated 107-mer version of the protein that includes only the TMDs does not co-localize with the other structural proteins when co-expressed and remains in the ER. Surprisingly, a deletion of residues 118-200 in the cytosolic domain that conserves the last C-terminus 21 residues recovers the ERGIC-Golgi localization when the other proteins are present. This indicates that this C-terminus tail is key for interacting with viral structural proteins, thus allowing virion assembly in the Golgi compartment.

**Póster 41**

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### **Structural and molecular insights in the phosphotransference mechanisms of novel fungal phosphorelay systems**

Asp-His phosphorelay systems are signal transduction systems comprised by a sensor hybrid histidine kinase (hyHK) and an effector response regulator (RR) present in every realm of life except from animals that enable rapid responses to changes in the environment (1,2). In these systems, upon sensing, the hyHK autophosphorylates and transfers the phosphoryl group first into a conserved Asp residue in a receiver domain (REC) present in its sequence, then shuffles it to a Histidine-containing phosphotransferase (HPt), and lastly to the Asp in the REC domain of a RR which exerts its function (3). In fungi, there are several hyHKs and at least two conserved RRs, Skn7 and Ssk1, which connect with high-osmolarity glycerol (HOG1) mitogen-activated protein (MAP) cascade (4) but *Saccharomyces cerevisiae* is the only characterized system that contains solely one hyHK (5). This highlights the importance of HPt in connecting the signals upstream and downstream and raises the question about the effect of the recognition specificity of HPt and the other proteins in the conservation of the signal.

To gain insights of the recognition mechanism, we produced several REC domains of several hyHKs and the HPt of the thermophile *Chaetomium thermophilum* (Cth-HPt) as well as the HPt of fungal pathogen *Candida albicans* (Cal-HPt) and *Candida auris* (Cau-HPt). Then, we performed phosphorylation and phosphotransference experiments using native PAGE. Also, we performed crystallization experiments obtaining the complex structure of Cth-HPt with the REC domain of hyHK CTHT\_E (RECCTHT\_E) from *C. thermophilum*, as well as the REC domains from hyHK CTHT\_F (RECCTHT\_F) from *C. thermophilum* and hyHK Sln1 from *C. albicans* (RECCal\_Sln1). The complex structure revealed three major anchor points of interaction between the REC and HPt (loops α1β1, α4β4 and α5β5). Interestingly, our data has allowed to understand the recognition mechanism between HPt and REC domains from hyHK, observe an uncoupled Y-T mechanism and propose that hyHKs exploit transient phosphotransfer to HPt demonstrated by the lack of a Leu-Thr switch to stabilize phosphoryl groups at the active site.

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## Póster 42

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### Protein Expression Systems.

Protein expression systems play a pivotal role in biotechnology and biomedical research by enabling the production of recombinant proteins with diverse applications. This abstract provides an overview of protein expression systems, their significance, and their impact on various fields of science and medicine.

Protein expression systems serve as powerful tools to produce proteins of interest, allowing scientists to investigate their structure, function, and therapeutic potential. These systems involve the transfer of genetic material encoding the target protein into host organisms or cell lines, which subsequently synthesize and assemble the protein. Multiple expression systems have been developed, each with unique features and advantages. Choosing the appropriate expression system based on the protein's characteristics, desired modifications, and downstream applications is crucial.

This work highlights the two primary classes of protein expression systems: prokaryotic and eukaryotic. Prokaryotic systems, such as *Escherichia coli*, offer simplicity, high protein yields, and cost-effectiveness, making them popular for industrial-scale production. Eukaryotic systems, including yeast, insect cells, and mammalian cells, provide the advantage of post-translational modifications and proper folding, critical for the production of complex proteins.

In summary, protein expression systems are invaluable tools for producing recombinant proteins and advancing biotechnological research. Their diverse applications and continuous advancements make them indispensable in numerous scientific disciplines, paving the way for new discoveries and innovations in medicine and industry.

**Póster 44**

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**miPEPs prediction in Solanum Lycopersicum**

MicroRNAs (miRNAs) are small regulatory RNAs that direct post-transcriptional gene silencing through cleavage or translational repression of target mRNAs. Their targets are mainly regulatory genes, so they are involved in several biological processes such as plant development and stress response (Liu et al., 2018). They are transcribed as larger primary transcripts (pri-miRNA), and recent findings revealed that short ORFs in them are translated into regulatory peptides called miRNA-encoded peptides (miPEPs) (Lauressergues et al., 2015; Sharma et al., 2020), which enhance the transcription of their pri-miRNA, leading to a stronger repression of the target gene. Some miPEPs have been found as useful tools in agronomy (Ormancey et al., 2021). Our objective was to create a database containing miPEPs derived from *Solanum lycopersicum* genome, aiming to exploit it for the development of novel strategies that optimize nitrogen and water use efficiency in a context of climate change. We used miRbase microRNA database, the Blast tool in Sol Genomics Network, BDPG: Neural network promoter prediction software and AlphaFold protein structure prediction software, to generate a database of all tomato miPEP sequences, along with additional relevant information for their utilization.

**Póster 45**

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**Targeting MCL 1 and BOK transmembrane interaction: membrane protein overexpression/recombinant expression and functional assay**

Permeabilization of the outer mitochondrial membrane by proapoptotic BCL-2 proteins is a crucial step for the induction of apoptosis. The interactions among pro- and anti-apoptotic members of the BCL-2 protein family play a critical role in determining the cell's fate by modulating this permeabilization. We have recently discovered that myeloid leukemia cell differentiation protein (MCL1), an anti-apoptotic member of the BCL2 family, interacts with

BCL2-related ovarian killer (BOK) through the transmembrane domain (TMD). To structurally characterize this interaction, our primary goal has been to set up the conditions for MCL1 and BOK expression and purification. Here, we show the results of optimized protein expression and purification protocols for these transmembrane proteins and preliminary results showing the inclusion of these proteins in nanodiscs.

## Póster 47

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## Perfil de miRNAs en suero de ratón asociado al diagnóstico/pronóstico de la enfermedad de Wilson.

La enfermedad de Wilson (EW) es un trastorno hereditario del metabolismo del cobre causado por mutaciones en Atp7b. Este gen codifica para una proteína transportadora del cobre; su mal funcionamiento provoca un fallo en la excreción biliar y la acumulación progresiva de este metal en el organismo, especialmente en hígado y cerebro. Dado que la EW es tratable con quelantes del cobre, es importante lograr un diagnóstico certero y temprano en los pacientes, ya que, en caso de no recibirlas a tiempo, se convierte en un trastorno con mal pronóstico. Estudios previos en nuestro laboratorio mediante secuenciación masiva de miRNAs circulantes en plasma han logrado identificar miRNAs que se encuentran significativamente desregulados en los pacientes, más concretamente los miR-122-5p, miR-192-5p y miR-885-5p. El objetivo del presente trabajo es evaluar la evolución de esta firma de biomarcadores diagnóstico en un modelo murino no tratado a lo largo del progreso de la enfermedad.

## Póster 48

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## Estudio de la interacción entre la proteína E de SARS-CoV-2 y la proteína antiapoptótica BclXL.

La proteína E (proteína de envoltura) es una de las proteínas estructurales del virus SARS-CoV-2. Esta proteína está implicada en la alteración de la homeostasis y del microambiente de la célula hospedadora, así como en procesos de apoptosis, inflamación y autofagia. La proteína BclXL es un miembro de la familia de proteínas Bcl-2, se localiza en la mitocondria y es una proteína antiapoptótica cuya función principal es promover la supervivencia celular.

Estudios previos en SARS-CoV muestran que BclXL ejerce un papel protector frente al efecto apoptótico inducido por E, por ello, se decidió comprobar si en SARS-CoV-2 se produce interacción entre ambas proteínas. Para ello, se recurrió a dos métodos, BlaTM y BiFC. BlaTM permite el estudio de interacciones homotípicas y heterotípicas entre dominios transmembrana en *E. coli*, mientras que BiFC (Bimolecular Fluorescence Complementation) hace posible el análisis de estas interacciones en cultivos celulares eucariotas. En ambos sistemas se observa la interacción heterotípica entre E y BclXL, demostrando que existe relación entre ambas proteínas en SARS-CoV-2.

### Póster 49

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## Análisis metabólico de levaduras hibridables con *Saccharomyces cerevisiae* en la producción de cerveza

La cerveza es una de las bebidas alcohólicas más consumidas a nivel mundial. Se espera que en los próximos años se produzca un aumento en el consumo de cerveza con bajo contenido alcohólico debido a la nueva tendencia de los consumidores de seguir comportamientos más saludables. Una nueva vía para su obtención es el uso de levaduras no convencionales que muestren una menor producción de alcohol. Para ello, es necesario realizar estudios metabólicos en búsqueda de nuevos cultivos que presenten una menor producción de etanol pero que, a su vez, tengan un buen perfil fermentativo y aromático que haga posible su uso a nivel industrial.

En nuestro grupo se ha realizado un estudio metabólico sobre más de 300 cepas de levaduras que representan diferentes subpoblaciones dentro de las especies del género *Saccharomyces* (*S. cerevisiae*, *S. kudriavzevii*, *S. uvarum*, *S. eubayanus*, *S. paradoxus*, *S. jurei*, *S. mikatae* y *S. arboricola*) y dentro de otros géneros de la familia *Saccharomycetaceae* como *Kazachstania* y *Naumovozyma*. En definitiva, levaduras genéticamente cercanas a *S. cerevisiae* que puedan ser potencialmente hibridables con esta misma. Las diferencias presentes en el consumo de los principales azúcares del mosto utilizado en la industria cervecera, la maltosa (~60%) y la maltotriosa (~20%), van a determinar las principales características del producto final. Se ha analizado el perfil

fermentativo de las levaduras en estas condiciones mediante la medida de la producción de CO<sub>2</sub> y de los principales metabolitos. Este estudio ha revelado la variabilidad existente entre las diferentes poblaciones, lo que ofrece una amplia variedad de levaduras que podrían ser candidatas a ser explotadas industrialmente. Actualmente, las más interesantes se están utilizando en nuestro laboratorio para la generación de híbridos con el objetivo de conseguir la reducción del contenido alcohólico, generar nuevos perfiles aromáticos o solucionar otros problemas de la industria cervecera.

## Póster 50

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### The posttranscriptional regulator AtC3H15 from *Arabidopsis* functions in metal homeostasis

Living organisms suffer different abiotic stresses, including that caused by limited micronutrient such as iron deficiency. To mitigate the effects of this type of stress, cells reserve the scarce iron available for essential processes. In the yeast *Saccharomyces cerevisiae*, under iron deficiency, certain tandem zinc finger (TZF) proteins regulate gene expression at the posttranscriptional and translational level of target mRNAs encoding non-essential ferroproteins. The molecular mechanism consists on binding to consensus sequences, denoted as ARE, which are present in the 3'-UTR region of the target mRNA. Specifically, the TZF ScCth2 protein binds to their target mRNA sequences, facilitating their degradation. The plant *Arabidopsis thaliana* has two TZF proteins, C3H14 and C3H15, homologous to *S. cerevisiae* ScCth1 and ScCth2. However, our data suggest that C3H14 and C3H15 do not play a key role in the *Arabidopsis* response to iron deficiency, although they could be involved in the homeostasis of other transition metals, such as copper and zinc. First, the expression of the C3H14 and C3H15 genes is up regulated by copper excess in the growth medium. Preliminary results conclude that the expression of C3H14 and C3H15 is not up-regulated by zinc or copper deficiency but their expression is induced in double zinc deficiency conditions. On the other hand, when *A. thaliana* seedlings with deregulated levels of the C3H14 and C3H15 are grown under copper deficiency, zinc translocation is altered. Moreover, in C3H15 OE adult plants the copper and zinc contents of protoplasts of these transgenic lines are reduced, which suggests that the metals are probably retained in the cell wall. Moreover, in C3H15 OE plants grown in hydroponic cultures under copper deficiency, the chlorophyll content and variations of chlorophyll fluorescence decreased compared to control plants, suggesting that accelerated senescence is taking place in these plants. Metal content in adult plants was analyzed in different tissues under copper deficiency and C3H15 OE plants showed increased copper content in roots whereas a reduced content was observed in seeds comparing to the WT plants. Taken together our data indicate that the TZF factor C3H15 function in plants is

related to metal homeostasis and that its previously described function in cell wall modification affect metal, mainly copper and zinc, translocation. These data point to C3H15 as a posttranscriptional regulator under copper excess that may counteract the effects on cell wall metal retention to favor metal translocation towards the seeds, the most important nutritional sink organ in plants.

## Póster 51

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### **Caracterización estructural del complejo multifactorial implicado en la iniciación de la traducción en eucariotas.**

Protein synthesis is carried out in a process called translation which can be divided into three stages: initiation, elongation and termination. Initiation is the highest regulated step in which the AUG start codon is recognized by the pre-initiation complex 48S with tRNA initiator (tRNA<sub>iMet</sub>) anticodon accommodated in the P site of the ribosome. In eukaryotes, this process requires the presence of different factors (eIFs), and many of them can interact and form a large complex called multifactor complex (MFC). This complex includes the factors eIF1, eIF3, eIF5 and the eIF2 bound to the GTP and the tRNA<sub>iMet</sub>. Some of them, like eIF3 and eIF5, act very early in the initiation process, which makes this complex an essential target in translation regulation. The resolution of the MFC structure by cryoelectron microscopy could be the key to getting more information about the location of their different factors and how they carry on their functions. So far, we have obtained a low-resolution model (25 Å) of the MFC using negative staining electron microscopy.

## Póster 52

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### **Identificación y caracterización tecnológica de aislados microbiológicos de masas madre.**

El pan es un alimento básico de consumo diario que aporta a la dieta un amplio abanico de nutrientes de alto interés para una alimentación saludable. En las últimas décadas, este concepto de vida saludable ha arraigado entre los consumidores, generándose una creciente demanda de productos “naturales”. Entre ellos, está bien aceptado que el uso de

masa madre, uno de los procesos biotecnológicos más antiguos, mejora el valor organoléptico y nutricional de los panes. Tradicionalmente, una masa madre es el resultado de la fermentación espontánea de una harina por parte de los microorganismos presentes en ella, levaduras, bacterias lácticas y hongos. Son comunidades simbióticas, que se van sucediendo a lo largo de la fermentación y que aportan a los productos elaborados con ellas, acidez, sabor y aroma diferencial, estructura peculiar de la corteza y migas, además de mejorar la calidad nutricional y alargar su vida útil.

Este Proyecto surge con la finalidad de utilizar masas madre para mejorar las propiedades organolépticas y nutricionales de productos de panadería. Durante este año, se ha llevado a cabo la determinación de parámetros químicos (pH, TTA, contenido en ácido láctico y acético y aromas), así como el aislamiento e identificación de la microbiota presente en estas masas madre. Se ha testado la tolerancia a estrés y la capacidad fermentativa de más de 100 aislados con el objeto de seleccionar aquellos que puedan formar parte de un cultivo iniciador. Nuestros resultados demuestran el potencial de la masa madre como fuente de estirpes de utilidad biotecnológica. La colección de aislados representa, además, un buen modelo para estudios evolutivos y para avanzar en nuestra comprensión de los mecanismos de resistencia a estrés en levaduras.

### **Póster 53**

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### **Descifrando el sistema arbitrium en los fagos Wbeta**

Los bacteriófagos temperados alternan en su ciclo vital los estados lítico – replicándose y lisando a la bacteria hospedadora – y lisogénico – integrándose en el cromosoma celular y replicándose con él. Recientemente se ha descrito que en diferentes familias de fagos cuyos hospedadores bacterianos pertenecen al filo Bacillota la decisión entre ambos ciclos la determina el sistema de quorum sensing denominado arbitrium, constituido por el clúster de genes aimR-aimP-aimX. Estos genes controlan la proteína cl, el represor del fago, cuyo silenciamiento por parte de AimX bloquea la entrada en lisogenia. Dicha inhibición desaparece cuando el péptido AimP impide la unión del regulador AimR al ADN y, con ello, la expresión de aimX. La existencia de sistemas arbitrium en múltiples familias de fagos y, más relevante, en otros elementos genéticos móviles, pone de manifiesto su amplia distribución, diversidad de mecanismos y su gran relevancia biológica. Esta diversificación ha llevado a dividir los sistemas arbitrium en hasta 10 clados, desconociéndose el funcionamiento exacto del mecanismo de acción de la mayor parte de ellos.

Con el fin de caracterizar las bases moleculares del mecanismo arbitrium en la familia de fagos Wbeta, un clado para el que aún no se tiene información, se han clonado, expresado y purificado varios pares AimX-cl pertenecientes a fagos de esta familia. Pretenden

analizarse sus interacciones moleculares y resolver sus estructuras tridimensionales para así profundizar en nuestra comprensión del sistema arbitrium y los mecanismos de decisión lisis-lisogenia. En la presentación se expondrán los avances conseguidos hasta el momento.

## Póster 54

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### **Mog1 protein might participate in Cell Division and Brugada syndrome**

Mog1 is a nuclear protein that binds Ran GTP and participates in protein import from the cytoplasm to the nucleus. This function is conserved in eukaryotic organisms from *Saccharomyces cerevisiae* to humans<sup>1 2</sup>. But more recently, Mog1 has been also implicated in mRNA transcription and export and in the establishment of two epigenetic marks; H2Bub1 and H3K4me3 in *S. cerevisiae*<sup>3</sup>.

In addition, mutations in human Mog1 are associated to Brugada syndrome, a ventricular arrhythmia responsible of approximately 20% of the cases of sudden death in patients without structural cardiopathies<sup>4</sup>. It was found that Mog1 physically interacts with the cytoplasmic loop II of Nav 1.5, the α subunit of the sodium channel type 5, in human cardiomyocytes suggesting that Mog1 promotes its transport to the plasma membrane<sup>5,6</sup>. Previous studies have pointed the utility of using yeast as a model system for studying ion channels associated to human diseases because of the homology of human and yeast ion channels<sup>7</sup>. This is the case of Cch1, a voltage gated calcium channel in yeast that we propose might be homologous to Nav 1.5. This is why we hypothesise that yeast Mog1 protein might interact with Cch1 so *S. cerevisiae* could be used as a model organism to study Brugada syndrome. As a first approach to study Mog1 and Cch1 interaction, we searched for genetic interactions by analysing the phenotype of yeast strains with deletions in CCH1, MOG1 or both genes from two genetic backgrounds (BY4741 and W3031A) and grown under different stress conditions. We found that MOG1 and CCH1 interact genetically under stress caused by exposure to NaCl and Sorbitol in both genetic backgrounds although the phenotype is more pronounced when using BY4741 strains. We also found genetic interaction between CCH1 and MOG1 genes when cells were grown at 37 °C (only BY4741).

On the other hand, in previous studies we found that yeast cells lacking MOG1 gene are 1.16 times bigger in size than wild type cells<sup>3</sup> which is a phenotype associated with cells having defects in cell cycle. There is also evidence for Mog1 contribution to mitotic spindle formation in HeLa cells through Ran GTP interaction<sup>8</sup>. Therefore, Mog1 could also participate in regulating yeast cell cycle. To answer this question, we synchronized cells lacking MOG1 gene at the metaphase-anaphase transition and compared the cell cycle profile to wild type cells. Cell cycle phases were followed by flow cytometry and

immunoblotting of G1 and G2 markers, finding no significant differences between wild type and Mog1 mutant cell cycles using these approaches.

## Póster 55

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### Tècniques per establir una xarxa d'interaccions entre dominis transmembrana de proteïnes Bcl2 virals i cel·lulars que permeten regular l'apoptosi en un context d'infecció viral pel virus Vaccinia

En organismes multicel·lulars, la mort cel·lular programada és crucial per al desenvolupament i homeòstasi dels teixits. Per la seua rellevància, requereix una estricta regulació, entre la qual un dels principals moduladors és la família de proteïnes coneguda com a Bcl2 (cBcl2). Aquestes proteïnes controlen la mort cel·lular programada mitjançant una interacció amb membres pro i antiapoptòtics de la mateixa família proteica Bcl2. A més, aquest procés es troba relacionat amb la defensa enfront de diversos trastorns, com algunes afeccions causades per patògens, en les quals, un control i eliminació eficients a menut requereixen l'apoptosi cel·lular. Els virus han desenvolupat diverses estratègies per modular la mort cel·lular programada, entre els quals es troba l'expressió d'anàlegs de les proteïnes de la família Bcl2, coneudes com a Bcl2 virals (vBcl2). S'ha demostrat que aquests membres cel·lulars poden regular l'apoptosi mitjançant interaccions proteïna-proteïna, entre les quals els dominis transmembrana (TMD) que presenten participen en la interacció dins la membrana. Es té evidència que el virus Vaccinia presenta una proteïna vBcl2 denominada F1L que pot interaccionar amb diverses cBcl2 pro i antiapoptòtiques per tal de regular la mort cel·lular programada enfront d'un estímul apoptòtic. Aquest esdeveniment evidencia la necessitat de desenvolupar tècniques per tal d'establir una xarxa d'interaccions entre les diverses proteïnes virals i cel·lulars. Així com poder esbrinar les relacions proteïna-proteïna que s'estableixen per regular aquesta apoptosis cel·lular en cada moment del cicle viral.

## Póster 56

Sorribes-Dauden, Raquel (1); Miró, Pilar (1); Romero, Antonia María (1); Peris, David (1,2); Martínez-Pastor, María Teresa (1,3) and Puig, Sergi (1).

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### Adaptation of wine yeasts to copper excess

Wine is the second most consumed alcoholic beverage worldwide, making the wine industry one of the main economic sectors in certain countries. Copper at trace levels is essential for life since it participates as a cofactor in many key biological processes, however, it is toxic for microorganisms when present in excess through reactive oxygen species (ROS) production. In ecological viticulture, CuSO<sub>4</sub> is normally used as a fungicide, which has caused copper to accumulate in soils up to very high levels. Copper excess can reach grape must slowing fermentation and altering the final quality of wine. *Saccharomyces cerevisiae* strains adapted to copper excess have an elevated copy number of CUP1 gene, which encodes for a copper-chelating metallothionein regulated by the transcriptional factor Ace1. In this line of research, our aim is to obtain copper-resistant strains from copper-sensitive strains of enological interest, in a non-GMO way. For this purpose, we have analyzed the growth of multiple *Saccharomyces* genus strains and some of their hybrids in media with high levels of copper. We have observed that non-*cerevisiae* strains are more sensitive to copper than *S. cerevisiae* strains, and hybrids between *S. cerevisiae* and *S. kudriavzevii* or *S. uvarum* have an intermediate phenotype. As previously reported, we have also noticed a correlation between copper resistance and CUP1 copy number. Moreover, through experimental evolution, we have obtained a copper-resistant strain and fully characterized single colonies from populations of two steps of the process. We found that at the final steps, there is a CUP1 copy number amplification and that growth in medium without copper addition is compromised. Thus, adaptation to copper is a complex process occurring mainly through CUP1 amplification and may affect growth in non-supplemented medium.

### Póster 57

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### Dual Regulation of Start Repressors: Balancing Levels and Function to Drive G1 Arrest during Cell Wall Stress.

Start is the main cell cycle checkpoint in *Saccharomyces cerevisiae*, where the cell irreversibly commits to enter in the cell cycle. At this point, yeast cells integrate internal and external signals with the Start control to decide whether to initiate or not a new round of cell division. Under normal conditions, Whi5 acts as the main transcriptional repressor of Start, while its functional paralog, Whi7, plays a minor role. However, Whi7 protein levels increase during cell wall stress, suggesting a more relevant role for Whi7 under this condition. In this work we investigate the molecular bases that determine the strength of Whi7 and Whi5 as Start transcriptional repressors during cell wall stress. We found that Whi7 is more unstable during cell wall stress, and that Whi7 degradation promotes cell cycle re-entry under this

condition. Consistently, the transient G1 arrest caused by cell wall stress depends on Whi7. Furthermore, we show that Whi7 binding to Start promoters specifically increase during cell wall stress. Interestingly, although Whi7 and Whi5 protein levels positively correlate with their ability to bind Start promoters, we found that protein levels are not the sole determinants of their strength to promote G1 arrest. Thus, Whi7 and Whi5 strength as Start repressors is influenced not only by their protein levels but also by other regulatory mechanisms that may confer to Whi7 specific function in stress. These findings highlight the complex regulation of Start under stress conditions and indicate that the interplay between Whi7 and Whi5 repressors is subject to multiple levels of control to ensure an appropriate response to environmental conditions.

## Póster 58

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## Paint4IRAS. Desarrollo de pinturas biocidas frente a virus y bacterias en entornos hospitalarios

Actualmente, las enfermedades nosocomiales y las infecciones relacionadas con la asistencia sanitaria (IRAS) suponen un gran problema de salud pública. El presente proyecto, Paint4IRAS, pretende implementar una solución tecnológica validada consistente en el desarrollo de una pintura con actividad frente a algunos de los virus y bacterias presentes en entornos hospitalarios y sanitarios. Mediante técnicas computacionales predictivas, se han podido seleccionar compuestos de origen natural activos frente a algunos de los agentes infecciosos más habituales en dichos entornos. Seguidamente, con tal de incorporar los principios activos (antimicrobianos y antivirales) a la pintura se aplicarán tecnologías de micro y nanoencapsulación. Estas tecnologías están diseñadas tanto para optimizar la compatibilidad y la actividad de los compuestos activos con las pinturas desarrolladas, como para que los compuestos sean liberados de forma controlada y así favorecer una mayor vida útil del producto final aplicado en los entornos sanitarios. Finalmente, se llevarán a cabo validaciones experimentales que confirmen los resultados obtenidos, tanto de los compuestos activos seleccionados (mediante ensayos in vitro para evaluar las actividades biocidas predichas) como de los productos formulados, evaluando su eficacia en entornos hospitalarios reales.

**Póster 59**

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**Mip6 role in transcriptional memory**

Cells are exposed, throughout their lives, to different changes in their environment and they must be capable of responding to them and reprogramming their genetic expression patterns in order to get adapted to these changes. Moreover, they often suffer several expositions to the same stimulus, and are able, in the second or subsequent expositions, to respond in a more efficient way to the stimulus. This is known as transcriptional memory.

In *Saccharomyces cerevisiae*, this transcriptional memory has been studied in the context of the expression of the GAL genes and INO1. One of the mechanisms that participate in the repression transcriptional memory (RTM) is the histone deacetylase complex Rpd3, which interacts physically, genetically and functionally with Mip6, a protein that participates in gene expression at different levels. In this work, we study, first, if there is a transcriptional memory upon heat shock and, in the case of confirmation, if Mip6 participates in the regulation of this memory.

Our results show that most of the genes present significant differences in the WT strain upon heat shock suggesting that transcriptional memory upon heat shock occurs in yeast cells. Interestingly, among the genes that are expressed faster when they were exposed to a previous stimulus, we find genes that code for proteins that belong to protein complexes with a role in chromatin modification or transcription. Furthermore, there is also a small percentage of genes which show memory patterns when the WT is compared to *mip6Δ*. The most represented family of genes showing these differences are those coding for ribosomal proteins. Thus, we conclude that Mip6 might participate in establishing the correct transcriptional memory pattern upon heat shock especially for ribosomal protein genes.

**Póster 60**

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**Establecimiento de nuevos sistemas de cultivo 3D basados en hidrogeles para la investigación en Hepatología.**

Para los estudios toxicológicos o la investigación *in vitro* de las enfermedades hepáticas tradicionalmente se han empleado modelos de cultivo celular hepático bidimensionales

(2D), que sin embargo no logran reproducir con exactitud la complejidad estructural y funcional del hígado. Con el objetivo de mejorar estas características, en los últimos años se han propuesto modelos experimentales tridimensionales (3D) basados en la utilización de soportes, chips o hidrogeles, entre otros. En nuestro grupo hemos estado desarrollando nuevos sistemas de cultivo 3D con la finalidad de generar modelos más realistas para la investigación en Hepatología. Para ello, hemos puesto a punto el cultivo de diferentes líneas celulares hepáticas (líneas de hepatoma HepG2 y HepaRG, y línea de hepatocitos inmortalizados Upcyte) en hidrogeles de distintos biomateriales. Para valorar la funcionalidad de las células en los modelos 3D, hemos recurrido a múltiples técnicas como la tinción con sondas fluorescentes, la medida de la liberación de enzimas intracelulares, el estudio de la expresión génica o la valoración de parámetros hepáticos clásicos como la capacidad ureogénica, la producción de albúmina o las actividades citocromo P450, entre otros. Mediante la utilización de estas técnicas hemos observado que la funcionalidad de las diferentes líneas celulares utilizadas mejora en los sistemas 3D. El establecimiento de sistemas 3D optimizados nos permitirá en el futuro generar cultivos con un fenotipo más cercano al del hígado y con mayor utilidad y relevancia para la investigación en Hepatología.

## Póster 61

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### **Characterization of the response to iron deficiency of yeast strains with altered mRNA decay**

Iron is an essential micronutrient for all eukaryotic organisms because it participates as a redox cofactor in a wide range of biological processes. During iron deficiency, the *Saccharomyces cerevisiae* RNA-binding protein Cth2 promotes a global metabolic rearrangement that aims to optimize iron utilization. Cth2 is a tandem zinc finger (TZF) protein that specifically binds to AU-rich elements within the 3' untranslated region of many mRNAs encoding iron-containing proteins to promote their degradation and repress their translation. Cth2 is a nucleocytoplasmic shuttling protein that does not contain any nuclear export signal and depends on binding to its mRNA targets to exit the nucleus. Here, we have characterized some natural yeast strains expressing a Cth2 protein with altered TZF which display growth defects in iron-deficient conditions. By using complementary approaches, we demonstrate that the CTH2-G195R allele encodes for a protein with defects in targeted mRNA binding and degradation that cause its accumulation in the nucleus. When expressed in a laboratory strain, this allele leads to a growth defect in iron-deficient conditions. However, correction of this mutation in the naturally iron deficiency-sensitive strains by CRISPR-Cas9 does not improve growth in low iron conditions, despite recovery

of Cth2 mRNA decay capacity. These results suggest that additional genetic changes contribute to the growth defect of these strains in iron-deficiency conditions.



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