III JORNADA DE SEGUIMIENTO DEL PROGRAMA DE DOCTORADO DE BIOTECNOLOGÍA AVANZADA

Viernes 29 de abril de 2022

Salón de Grados de la Facultad de Ciencias de la Universidad de Málaga

Organizadores:

Dra. Margarita Pérez
(Profesora Titular de Fisiología)

Dra. Tahia Fernández
(Profesora Contratada Doctora de Fisiología)

Dra. Carmen Beuzón
(Catedrática de Genética y Coordinadora del Programa)

PROGRAMA DE DOCTORADO BIOTECNOLOGÍA AVANZADA

50 ANIVERSARIO | UNIVERSIDAD DE MÁLAGA

9:30 Acto de Inauguración de la Jornada:
Dra. Magdalena María Martín. Directora de la Escuela de Doctorado.
Dr. Antonio Flores. Decano de la Facultad de Ciencias.
Dra. Carmen Beuzón. Coordinadora del programa de doctorado de Biotecnología Avanzada.

9:45 La Tesis Doctoral: ayudas, menciones y trámite:
Dra. Magdalena María Martín. Directora de la Escuela de Doctorado.
Dra. Lourdes Rubio. Subdirectora de Formación y Calidad.

10:15 Un ejemplo de carrera científica:
Dra. Tahia Fernández. Profesora del área de Fisiología.

11:00 Sesiones de seguimiento de doctorandos en Biotecnología Avanzada

11:00 Doctorando: Jordi Díaz (Directores: José Antonio Fernández y Lourdes Rubio)
Zostera marina NRT2 gene encoding a Na+- dependent high-affinity NO3- transporter.

11:15 Doctorando: José Antonio Duarte (Directora: María Catharina Merchante)
Looking for specialized ribosomes in plants. Characterization of the riboprotein families L10 and L24.

Café y visita de Posters (comedor de la 1ª planta del edificio I+D)

12:15 Doctorando: Carla Lavado (Directores: Cayo Ramos y Luis Rodríguez)
Role of global and specific regulators of Pseudomonas savastanoi pathovars as determinants of virulence and host specificity.

12:30 Doctorando: Juan de los Santos (Director: José Manuel Matés)
Metabolic changes upon GLS inhibition by CB-839 in glioma cell lines.

12:45 Doctorando: José Antonio Torres (Directores: Ana Rodríguez y Melissa García)
Biological activity of new toluquinol derivative with improved potential as an antiangiogenic drug.

13:00 Doctorando: Julia Vega (Directores: Félix D. López y José Bonomi)
Potential cosmeceutic use of extracts from Porphyra species (Rhodophyta).

13:15 Doctorando: Antonio López (Directores: Juan Suarez y Patricia Rivera)
Dietary administration of D-Chiro-inositol attenuates sex-specific metabolic imbalances in the 5xFAD mouse model of Alzheimer's Disease.

13:30 Entrega de premios a la mejor presentación y mejor póster científico y Clausura de la Jornada

Zostera marina NRT2 gene encoding a Na^+- dependent high-affinity NO_3^- transporter

Jordi Díaz-García[1], Lourdes Rubio[1] and José A. Fernández[1]

[1]*Department of Botany and Plant Physiology, University of Málaga, Málaga, Spain.*

Seagrasses are the unique vascular plants that colonize the marine environment regaining functions to thrive in 0.5M Na^+, pH (8.2) and low availability of N and P. *Zostera marina* was the first fully sequenced seagrass, which genome points out some adaptation mechanisms seems to be due to molecular changes of the same gene families rather than speciation of pre-existing genes. Different NRT2s and NPF6.3 proteins have been characterised as H^+-dependent high-affinity NO_3^- transporters operating at the plasma membrane. However, no molecular evidences have been found to support the Na^+-dependent high-affinity NO_3^- uptake described in *Z. marina*. To unveil the NO_3^- uptake mechanism that evolved to use Na^+ as driving ion instead of H^+ we analyzed the expression levels of *ZosmaNRT2*; *ZosmaNAR2* and *ZosmaNPF6.3*, the only nitrate high-affinity transporter genes identified in *Z. marina* genome. Then, we investigated the NO_3^- transport capacity, kinetic and whether these transporters use Na^+ as driving ion by functional complementation in *Hansenula polymorpha Δynt1* mutant. Relative expression pattern shows that *ZosmaNAR2*, coding the ZosmaNRT2 partner protein, is the most expressed gene in natural seawater and is the main target under low NO_3^-. Functional complementation assays showed that ZosmaNRT2-expressing strains (Δynt1::NRT2 and Δynt1::NRT2::NAR2) were able to uptake NO_3^- only in the presence of Na^+, showing saturation kinetics regardless of the external pH. Either at pH 6.3 or 8.3, Δynt1::NRT2::NAR2 showed a higher NO_3^- uptake efficiency, this is twice Vmax (≈0.12 *vs* ≈0.05 $nmolNO_3^-{\cdot}min^{-1}{\cdot}mgcells^{-1}$) and almost five-times lower Km (≈5 *vs* 24 µM NO_3^-), than the Δynt1::NRT2. Yeasts expressing ZosmaNPF6.3 failed to uptake NO_3^- below 5 µM and in alkaline media, supporting its proton dependence and a non-suitable affinity for the marine environment. These results strongly indicate that the Na^+-dependent high-affinity NO_3^- uptake described in *Z. marina* is mediated by the two-component ZosmaNRT2::ZosmaNAR2, being the first Na^+-dependent high-affinity nitrate uptake mechanism to be functionally characterised from a vascular plant.

Looking for specialized ribosomes in plants. Characterization of the riboprotein families L10 and L24

José Antonio Duarte Conde[1], Gemma Sans-Coll[1], and Catharina Merchante[1]

[1]*Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga – Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Dpto. Biología Molecular y Bioquímica, UMA, Málaga, Spain.*

Introduction: Translation and its regulation play an important role in plant adaptation but ribosomes have been considered passive molecular machines in this process. This view is changing as studies are showing evidence for their active role in translational regulation in mammals and bacteria. The likelihood of ribosomal specialization is even higher in plants, with up to seven paralogs per family of ribosomal proteins in Arabidopsis and some hints pointing towards differential roles for the different paralogs. However, whether this heterogeneity allows for the selective translation of specific mRNAs under certain conditions has yet to be demonstrated. Our research tries to answer this question focusing on two families of ribosomal proteins, RPL10 and 24 which share characteristics that make them good candidates to look for paralog specialization, as the two of them are composed of multiple genes (A, B, and C) that are ubiquitously expressed. In addition, specific functions have been described for at least one paralog of each family.

Objectives: To determine whether the different paralogs of the ribosomal protein families PRL10 and RPL24 are involved in the selective translation of specific mRNAs and thus, play a role in adaptation.

Results: Our research shows that Arabidopsis mutants of different RP paralogs display phenotypical differences in both control and abiotic stress conditions, and we have been able to determine that, within the same family, some paralogs are necessary for the translation of uORF-containing transcripts while others are dispensable. We are performing RNA-seq experiments of both total and polysomal RNA to analyse the mRNA population that is being translated in each mutant and see if we can assign the translation of certain mRNAs to specific paralogs.

Conclusions: Our results suggest that the different paralogs of the riboprotein families L10 and L24 could be performing specialized functions.

Role of global and specific regulators of Pseudomonas savastanoi pathovars as determinants of virulence and host specificity.

Carla Lavado-Benito C[1,2], Martínez-Gil M[1,2], Santamaría-Hernando S[4], Murillo J[3], López-Solanilla E[4], Rodríguez-Moreno L[1,2], Ramos C[1,2]

[1]Área de Genética, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain. [2]Departamento de Microbiología y Protección de Cultivos, Instituto de Hortofruticultura Subtropical y Mediterránea «La Mayora», Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Málaga, Spain. [3]Institute for Multidisciplinary Research in Applied Biology, Universidad Pública de Navarra, Navarra. Spain. [4]Center for Plant Biotechnology and Genomics (CBGP-UPM-INIA), Madrid. Spain.

Pseudomonas savastanoi is a phytopathogenic bacteria, belonging to the *Pseudomonas syringae* complex, causing knots on a wide range of woody hosts. *P. savastanoi* includes 5 pathovars isolated from different woody hosts: pv. savastanoi, pv. nerii, pv. fraxini, pv. retacarpa and pv. mandevillae, isolated from olive, oleander, ash, broom and dipladenia, respectively. Infection of different hosts by *P. savastanoi* require the expression regulation of different virulence factors to facilitate its adaptation into the new environment. The aim of this work was to analyze the role of a global and specific regulators of *P. savastanoi* pathovars as determinants of virulence and host specificity.

In this sense, one of the main mechanisms for global regulation in bacteria is the two-component regulatory system GacS/GacA. We generated a *gacA* deletion mutant in Psv strain NCPPB 3335, whose transcriptomic profile was further analyzed using a massive RNA sequencing (RNA-seq) strategy. The bioinformatic analysis of RNA-seq data showed that the Psv GacS/GacA system regulates a large number of genes, including some virulence factors already described. Furthermore, this transcriptomic analysis was complemented with different phenotypic assays, such as plant virulence assays, hypersensitive response induction, foliar adhesion assays and type III effector translocation. All these results indicate that the GacS/GacA system participates in the regulation of virulence factors in Psv NCPPB 3335.

Based on a previous comparative genomic analysis carried out among different pathovars of *P. savastanoi*, we identified an 8723 pb genomic region that was present in all the strains belonging to the *P. syringae* complex but absent in the strains belonging to the pv. mandevillae, and two closely phylogenetic related strains of the pv. nerii. This genomic region contains two genes encoding for methyl chemotaxis proteins (MCP1, MCP2) and 6 chemotaxis genes (*cheY, cheA, cheB, cheD, cheR, cheW*). To analyze the functional role of this region, we generated knockout mutants for most of the genes in two different strains, the Psn ESC23 and *P. syringae* pv. tomato DC3000 (Pto DC3000), pathogenic in a woody and herbaceous host, respectively. These mutants were analyzed through plant virulence, swarming-type mobility, and chemotaxis assays. To identify the molecule that activates this chemotaxis cluster, the extracellular domain of the MCP2 was purified and used for specific binding substrate by thermal shift assay. Results obtained indicate that two of the chemotaxis cluster genes are involved in swarming mobility and virulence in Pto DC3000.

Metabolic changes upon GLS inhibition by CB-839 in glioma cell lines

Juan De los Santos-Jiménez[1,3], Tracy Rosales[2], Bookyung Ko[2], Javier Márquez[1,3], Ralph J. DeBerardinis[2] and José M. Matés[1,3].

[1]Departamento de Biología Molecular y Bioquímica, Canceromics Lab Facultad de Ciencias, Universidad de Málaga. [2]Children's Research Institute, University of Texas Southwestern Medical Center. [3]Instituto de Investigación Biomédica de Málaga (IBIMA-Plataforma BIONAND)

Introduction: Many tumors use Gln for both energy generation and biosynthesis. Glutaminases catalyze the first step of glutaminolysis by converting Gln into glutamate and ammonia in the mitochondria. In humans, two genes encode for glutaminases: *GLS* and *GLS2*. *GLS* is considered as a tumor-promoting gene. In glioma, GLS enzymes are usually overexpressed. We treated three human glioblastoma cell lines with the GLS inhibitor CB-839 and performed untargeted metabolomics and isotope tracing using U-13C-labeled Gln and 15N-labeled Gln in the amido group to ascertain the metabolic fates of Gln carbon and nitrogen.

Objectives: To examine the metabolic consequences of inhibiting GLS activity in glioma cells.

Results: CB-839 depleted tricarboxylic acid cycle (TCAC) intermediates in the three cell lines assayed. This result was also confirmed by a lower labeling from U-13C- Gln in these metabolites. Metabolomics showed an accumulation of the *de novo* purine biosynthesis intermediates IMP and/or AICAR, and a decrease in UMP, while 15N-Gln tracing showed a decreased labeling in AMP, GMP, UMP and CTP in T98G treated with CB-839. Finally, metabolomics showed higher levels of trimethyllysine and, in T98G, a 22-fold increase in 5-methyl-cytosine.

Discussion: Although metabolomics showed lower levels of TCAC metabolites in LN229, T98G and U87MG treated with CB-839, T98G showed the largest decrease and U87MG showed smaller differences. This reflects differential dependencies of glioblastoma cells on GLS activity for maintaining levels of TCAC metabolites. CB-839 also decreased nucleotide biosynthesis, most likely due to aspartate depletion, and apparently increased methylation in nucleotides and proteins, noted by elevated levels of 5-methyl-cytosine in T98G, and trimethyllysine in all three cell lines. These metabolites likely arise from degradation of DNA and proteins, including histones. These effects could result from reduced activity of α-KG-dependent demethylases, which use α-KG as a substrate and are inhibited by succinate. Accordingly, the α-KG/succinate ratio was significantly decreased in CB-839 treated cells.

Biological activity of new toluquinol derivative with improved potential as an antiangiogenic drug

José Antonio Torres-Vargas[1], Iván Cheng-Sánchez[2], Beatriz Martínez-Poveda[1], Miguel Ángel Medina[1,3], Francisco Sarabia[2], Melissa García-Caballero[1], Ana R. Quesada[1,3]

[1]Department of Molecular Biology and Biochemistry, University of Malaga, Andalucía Tech. [2]Department of Organic Chemistry, University of Malaga, Andalucía Tech. [3]U741 (CB06/07/0046) CIBERER and IBIMA, Malaga, Spain.

Angiogenesis, the formation of new blood vessels from other pre-existent ones is tightly regulated under physiological conditions by a balance of activators and inhibitors. On the contrary, a sustained angiogenesis is related to many pathological conditions, including ophthalmic disorders, cutaneous diseases, inflammation and cancer, among others. For this reason, angiogenesis inhibition has attracted broad attention in the field of pharmacological research. Encouraged by our previous findings that toluquinol exhibited an interesting antiangiogenic activity, we have explored the effects of structural modifications of this natural compound in order to develop improved drug candidates. Our results indicate that some derivatives in which the methyl group was replaced by another substituent, could keep the antiangiogenic activity, whereas exhibiting a lower cytotoxicity in vitro. Here, we describe the activity of a promising toluquinol derivative, (*E*)-2-(3-methoxyprop-1-en-1-yl) benzene-1,4-diol, prepared by mean of a Suzuki coupling reaction, which allowed to replace the methyl group by the vinyl-type substituent. Our data indicate that this compound either keeps or improves the antiangiogenic activity of toluquinol, as demonstrated by a number of in vitro assays (endothelial cell survival, proliferation, migration and tube formation) and in vivo models (chick chrorioallantoic membrane vascularization and murine corneal neovascularization). Interestingly, the toxicity of this new compound was lower than that of toluquinol, as demonstrated by means of *in vitro* studies carried out with transformed and non-transformed cells, and by *in vivo* studies performed in zebrafish embryos. Moreover, our results suggest that the mechanism of action of this compound could be related not only to its ability to block the VEGF-induced VEGFR2 autophosphorylation and the subsequent Akt and ERK downregulation, but also to the induction of apoptosis and the increase in the oxidative stress. The biological activity of this new angiogenesis inhibitor, along with its lower undesired toxicity, suggest an improved potential of this new toluquinol derivative as a drug candidate for angiogenesis-related diseases.

Potential cosmeceutic use of extracts from Porphyra species (Rhodophyta)

Julia Vega[1], José Bonomi-Barufi[2], Félix L. Figueroa[1]

[1]Universidad de Málaga, Instituto andaluz de Biotecnología y Desarrollo Azul (IBYDA), Departamento Ecología y Geología, Málaga, España. [2]Phycology Laboratory, Botany Department, Federal University of Santa Catarina, Florianopolis, SC, Brazil

Introduction: Species from the genus *Porphyra, Pyropia* or *Neopyropia* (Bangiales, Rhodophyta) live in the upper part of the intertidal zone exposed to extreme environmental conditions, such as: high solar radiation, hydrodynamism and temperature, salinity changes or desiccation. These species accumulate a wide diversity of antioxidant and UV-screen compounds as protecting mechanism against environmental stress, like mycosporine-like amino acids (MAAs). MAAs are nitrogenous molecules with low molecular weight (<400Da) with UV-screen and antioxidant properties.

Objetives: (i) Analyze different bioactivities (e.g. antioxidant, anti-photoaging or photoprotection) of extracts from different *Porphyra* species. (ii) Study the effects of UVA-Blue radiation and nitrate on the internal composition of *Neopyropia leucosticta* (focusing on MAAs) grown under laboratory conditions. (iii) Purification of MAAs from *Porphyra columbina* by using liquid-liquid chromatography.

Results: *Porphyra* extracts present high antioxidant activity (measured through ABTS and DPPH methods), reaching values of 15-20μM Trolox equivalent (TE) g-1 DW. The content of MAAs in *Porphyra* is one the highest reported in red algae (7-10mg g-1 DW). The extracts also showed capacity to inhibit collagenase activity but not elastase activity (both enzymes are related to structural damage of dermic proteins, that cause photo-aging). Despite *Porphyra* is known to present constitutive amounts of MAAs, an increase of MAAs content under UVA-Blue radiation and high nitrogen supply, was observed. The two main MAAs presented in *Porphyra* (porphyra-334 and shinorine), identified by HPLC and MS, were successfully isolated using high performance counter-current chromatography (HPCCC).

Conclusions: The red algae *Porphyra* is a good candidate to be used in cosmeceutical industry due to its high production of MAAs and culture possibilities. Different beneficial properties for the skin have been found in *Porphyra* extracts with potential photoprotection capacity to be used as UV-screen substance with antioxidant and anti-photoaging properties.

Dietary administration of D-Chiro-inositol attenuates sex-specific metabolic imbalances in the 5xFAD mouse model of Alzheimer's Disease

Antonio J. López-Gambero[1,2,3], Beatriz Pacheco-Sánchez[1], Cristina Rosell-Valle[1], Dina Medina-Vera[1,3,4,5], Juan Antonio Navarro[1,2,4], María del Mar Fernández-Arjona[1,2], Marialuisa de Ceglia[1,2], Carlos Sanjuan[6], Vincent Simon[7], Daniela Cota[7], Patricia Rivera[1,2], Fernando Rodríguez de Fonseca[1,2,*] and Juan Suárez[1,4,8,*]

[1]Instituto de investigación Biomédica de Málaga-IBIMA, Málaga, Spain. [2] UGC Salud Mental, Hospital Regional Universitario de Málaga, Málaga, Spain. [3] Universidad de Málaga, Andalucia Tech, Departamento de Biología Celular, Genética y Fisiología, Málaga, Spain. [4]Universidad de Málaga, Andalucia Tech, Facultad de Medicina, Málaga, Spain. [5]UGC Corazón, Hospital Universitario Virgen de la Victoria, Málaga, Spain. [6]EURONUTRA S.L, Parque Tecnológico de Andalucía, Campanillas, Spain. [7]University of Bordeaux, INSERM, Neurocentre Magendie, Bordeaux, France. [8]Departamento de Anatomía Humana, Medicina Legal e Historia de la Ciencia, Universidad de Málaga, Málaga, Spain.

Introduction: Increasing evidence shows that hypothalamic dysfunction, insulin resistance, and weight loss precede and progress along with the cognitive decline in sporadic Alzheimer's Disease (AD) with sex differences.

Objectives: This study aimed to determine the effect of oral dietary administration of D-Chiro-inositol (DCI), an inositol used against insulin resistance associated with polycystic ovary, on the occurrence of metabolic disorders in the transgenic 5xFAD mouse model of AD (FAD: Family Alzheimer's Disease). DCI was administered from 6 to 10 months of age to male and female 5xFAD mice and control (non-Tg) littermates. Energy balance and multiple metabolic and inflammatory parameters in the hypothalamus, liver and plasma were evaluated to assess the central and peripheral effects of DCI.

Results: Results indicated that weight loss and reduced food intake in 5xFAD mice were associated with decreased neuropeptides controlling food intake and the appearance of a pro-inflammatory state in the hypothalamus. Oral administration of DCI partially restored energy balance and hypothalamic parameters, highlighting an increased expression of *Npy* and *Agrp* and female-specific downregulation of *Gfap* and *Igf1*. DCI also partially normalized impaired insulin signaling and circulating insulin, GLP-1, and GIP deficiencies in 5xFAD mice. Principal component analysis of metabolic parameters indicated the presence of a female-specific fatty liver in 5xFAD mice: DCI administration reversed hepatic fat accumulation, β-oxidation, inflammation and increased GOT and GPT levels.

Conclusions: Our study depicts that metabolic impairment along with the cognitive decline in a mouse model of AD, which is exacerbated in females, can be ameliorated by oral supplementation with insulin-sensitizing DCI.

NTMC2T5 protein family: newly identified ER-Chloroplast contact site proteins involved in abiotic stress.

Carolina Huércano[1] and Noemí Ruiz-López[1]

[1]. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Málaga, Spain.

In plants, fatty acid synthesis takes place at chloroplasts, and they are assembled into glycerolipids and sphingolipids at the endoplasmic reticulum (ER). Then, the newly synthetized lipids in the ER are delivered to chloroplast through lipid transport proteins (LTP) via a nonvesicular pathway. Generally, these LTP are localized in membrane contact sites (MCS), which are microdomains where membranes of two different organelles are closely apposed. Some LTP at MCS contain specific domains, as the synaptotagmin-like mitochondrial lipidbinding (SMP) domain. To our knowledge, no protein has been identified to be directly involved in transfer of lipids between ER and Chloroplast. The objectives of this thesis are the identification of SMP proteins that would be involved in the lipid transport in ER-Chloroplast MCS and elucidation of their roles in abiotic stress conditions in plants. We have studied the occurrence of SMP proteins in *A. thaliana* and S*. lycopersicum* by searching remote orthologs of human E-Syt1 (SMP protein). By using transient expression in *N. benthamiana* leaves and confocal microscopy, we have identified the NTMC2T5 family with two homologs in *A. thaliana* and only one in *S. lycopersicum* that are anchored to the chloroplast and interacting with the ER (ER-Chloroplast MCS). Additionally, when we overexpress them, clustering of chloroplasts around the nucleus occurs. Also, *Arabidopsis* double *knock-out* mutant for these proteins showed less chloroplasts attached to the nucleus in leaves at control conditions. Moreover, *Arabidopsis* simple mutants have shown lower germination rates in media supplemented with NaCl and lower rates of expanded cotyledons in media supplemented with ABA.
These results show the first identified proteins localized in ER-Chloroplast MCS which might have a role in lipid transfer, specifically they are interacting with the ER that form the nuclear envelope. Our results suggest they might be involved in abiotic stress signalling through an ABA-dependent pathway.

Stronger in weakness: Reinforcing the plant cell wall during a stress

Francisco Percio[1]; Vitor Amorim-Silva[1]; Miguel A. Botella[1]

[1]Departamento de Biología Molecular y Bioquímica, Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora," Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC), Universidad de Málaga, Málaga, Spain

Cellulose is the most abundant organic compound of all biomass on Earth. It comprises 150 gigatons of carbon (Gt C) of a total ≈550 Gt C of biomass present on Earth. In addition to its societal and biological importance, it plays a fundamental role in plant development and defence, so it's essential to elucidate the regulation of its biosynthesis to improve crops tolerance to biotic and abiotic stresses. Our group has shown that tetratricopeptide-repeat thioredoxin-like (TTL) proteins play a role in the stabilization of cellulose synthase complexes (CSC) after salt stress, but further studies are needed to elucidate the complex regulation of this process.
The main objectives of this project are (1) to identify receptors that detect changes in cell wall, triggering the CSC response to adapt to the new conditions; and (2) characterize the role of BIK1 (a receptor like cytoplasmic kinase involved in plant immunity) as an intermediate for signalling from the cell wall to the TTLs that leads to their relocalization to the CSC.
As a first objective, I have generated lines to analyse the genetic interaction between *TTLs* and the receptor like kinases *Theseus* and *Feronia*. As a second objective, I have (1) confirmed the role of BIK1 in cellulose biosynthesis by analizing *bik1* mutants in the presence of the cellulose synthesis inhibitor isoxaben; (2) investigate the genetic interaction between *TTL* and *BIK1* genes; and generated lines that will allow to study the effect of *BIK1* in the dynamic localization of TTL3 and the CSC after a stress using spinning disk, TIRF microscopy and microsomal fractionation analysis.
This project will bring new insights in cellulose biosynthesis regulation and stablish a role for *BIK*1 and *TTLs* in abiotic stress responses.

Bioinformatics approaches applied to the phenotypic and transcriptomic analysis of rare diseases related to neuromotor impairment

José Córdoba Caballero[1,3], Pedro Seoane Zonjic[1,3,4], and Juan Antonio García Ranea[1,3,4]

[1]Departamento de Biología Molecular y Bioquímica, Universidad de Málaga, Málaga, España. [2]Instituto de Investigación e Innovación Biomédica, Cádiz, Spain. [3]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Madrid, Málaga, Spain. [4]Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain.

Introduction: Rare diseases are pathologies that have a low frequency in the population. In Europe, diseases are considered rare if they affect 1 person in at least 2 000. This low prevalence makes the molecular characterization of these diseases a challenging task. Furthermore, diagnosis can be very difficult due to lack of knowledge. Nowadays, high throughput sequencing technologies have been essential to development of precision medicine. In addition, these technologies have allowed us to find expression patterns caused by the disease and the genetic variants responsible for them.

Objectives: The objectives of this thesis are i) the implementation of RNAseq analysis to characterize the molecular mechanisms involved in rare disease and ii) to aid the correct diagnosis of patients with rare disease, through the application of disease-phenotypes network approaches.

Results: In our laboratory, we work on the molecular characterization of rare diseases related to neuromotor impairment, such as PMM2-CDG and Lafora. In these cases, we analysed RNAseq and miRNAseq data with our in-house tool ExpHunter that performs differential expression and co-expression analysis. Our results showed an alteration of the formation and composition of the basement membrane for PMM2-CDG and confirmed previous findings related to microglia-astrocyte cross talk in neurodegeneration for Lafora disease. To help in the diagnosis of rare disease patients, we build disease-phenotypes networks from diverse sources. Then, we analysed the co-occurrence of the phenotypes across diseases to group them into clusters. For these clusters, we can identify potential causal genes and associate them with disease. We found 6793 phenotype groups that belong to 4026 diseases in the OMIM database.

Conclusion: The presented work shows how our expression analysis methodology can help in rare disease research. Furthermore, the co-occurrence phenotype group analysis can help in rare disease diagnosis and identifying the genes responsible for the disorder.

Gene expression analysis in tomato introgression lines give new insights on genetics of fruit cuticle deposition

Juan Carlos Mateos del Amo[1], Eva Domínguez Carmona[1], Fernando Gallardo Alba[2], Rafael Fernández-Muñoz[1]

[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM, UMA-CSIC), Málaga, Spain. [2]Departamento de Biología Molecular y Bioquímica, Universidad de Málaga (UMA), Málaga, Spain.

Introduction: Cuticle is the outermost layer that covers the aerial organs of the plant and protects it from both biotic and abiotic stresses. Due to its unique properties, the modification of the cuticle can be considered for in crop improvement and protection and may led to novel material development such as bioplastics.

Objectives: Despite the rising interest in describing cuticle properties and its potential application as a material, the current knowledge on the genes responsible for cuticle deposition during plant organ development is still fragmentary. In tomato, two regions in the chromosome 3 were previously identified as QTLs for fruit cuticle mass (cm3.1 and cm3.2). By using a Recombinant Inbred Line (RIL) population of an interspecific cross involving a wild-relative species and a tomato cultivar (Moneymaker, MM) that differ greatly in cuticle characteristics, it was observed that in a newly-created Introgression Lines (IL) harboring the wild-species *Solanum pimpinellifolium* chromosome-3 region containing *cm3.1* and *cm3.2* had less cuticle mass than those containing the cultivated species S*. lycopersicum* QTL alleles. Aiming to find candidate genes for these QTLs, gene expression of annotated genes situated in the two QTL-peak regions was analyzed by RT-qPCR of RNA extracts from peel of fruits in four stages of development in both the IL sp3-3 line, containing both cm3.1 and cm3.2 S. *pimpinellifolium* alleles in the genetic background of MM, and in MM cultivar.

Results: The genes contained in both QTLs were studied and the gene expression for 42 of them were analyzed. The genes ranged from different putative functions, including membrane synthesis, cell structure development, different metabolic routes, and signaling. After bioinformatic analysis, the genes with the biggest differences in their expression profiles were re-analyzed using two shortened ILs obtained from sp3-3 harboring isolated *cm3.1* and *cm3.2* regions, and avoiding a possible interference in cuticle deposition. from other known QTLs which co-localize in the near zone to *cm3.2*.

Conclusions: The results obtained from this second analysis were consistent with the previous one. Work is in progress to identify the possible candidate genes for the cuticle deposition within cm3.1 and cm3.2 regions to elucidate the genetic mechanisms underlying the observed differences in cuticle deposition in tomato cultivars.

Effects of exogenous application of methyl jasmonate and salicylic acid in avocado physiology and control of white root rot disease

A. Moreno-Pérez[1], G. Moreno-Ortega[2], E. Martínez-Ferri[2], C. Pliego[1]

[1]Department of Genomics and Biotechnology, Unidad Asociada IHSM-IFAPA, IFAPA Málaga, Málaga,SPAIN. [2]Department of Crop Ecophysiology, Unidad Asociada IHSM-IFAPA, IFAPA Málaga, Málaga, SPAIN.

White root rot (WRR) disease caused by the soilborne pathogen Rosellinia necatrix is one of the main biotic stresses affecting avocado in southern Spain. Elicitors induce defense mechanisms in plants similar to defense responses induced by pathogens. Their recognition triggers several signal transduction pathways resulting in the induction of plant defense genes which modulate different biochemical and physiological responses. Methyl jasmonate (MeJA) and salicylic acid (SA) are two of the most studied elicitors because of their relevance mediating plant response to abiotic and biotic stresses. The aim of this research was to evaluate the effect of exogenous application of MeJA and SA in avocado plants through their physiological response and control of white root rot disease caused by *R. necatrix*. Plants of four-years-old 'Dusa' avocado rootstocks, susceptible to *R. necatrix*, were sprinkled weekly with MeJA or SA 5mM solutions. Following six elicitations, plants treated with SA resulted in an increment in assimilation rates (AN) and stomatal conductance (gs) indicating an effect of SA at the stomatal control level. No significant differences were observed in relative chlorophyll content (SPAD index) or leaf water potential between treatments. Exogenous application of MeJA showed an increase in nonphotochemical quenching of fluorescence (NPQ and qN) suggesting the activation of energy dissipating mechanisms related to oxidative stress. MeJA decreased leaf water content (LWC) and leaf relative water content (RWC) in consonance with an increase in the specific leaf mass area (LMA), suggesting an increase in the leaf thickness/density, previously related to plant adaptation to stressful environments. Inoculation experiments revealed a positive effect of MeJA in control of WRR disease, being able to reduce symptoms development approximately 30% respect to untreated control plants. These results support the use of elicitors as an environmentally friendly strategy to control avocado diseases.

Dietary use of the microalga Chlorella fusca improves growth and digestive functionality in thick-lipped grey mullet (Chelon labrosus, Risso 1827) juveniles

Jorge García-Márquez[1], Gabriel Acién[2], Félix L. Figueroa[3], Francisco Javier Alarcón[4], Salvador Arijo[1], Roberto Teófilo Abdala-Díaz[3]

[1]Departamento de Microbiología, Facultad de Ciencias, Instituto Andaluz de Biotecnología y Desarrollo Azul (IBYDA), Universidad de Málaga, Ceimar-Universidad de Málaga, Málaga, Spain. [2]Departamento de Ingeniería Química, Universidad de Almería, Ceimar-Universidad de Almería, Almería, Spain. [3]Departamento de Ecología y Geología, Facultad de Ciencias, Instituto Andaluz de Biotecnología y Desarrollo Azul (IBYDA), Universidad de Málaga, Ceimar-Universidad de Málaga, Málaga, Spain. [4]Departamento de Biología y Geología, Universidad de Almería, Ceimar-Universidad de Almería, Almería, Spain.

In recent years, a clear emphasis has been placed on replacing fishmeal and fish oil in aquafeeds with other alternative ingredients, including algae, particularly in low trophic omnivorous fish species. This work aimed at evaluating the effects of moderate dietary supplementation with the green microalga *Chlorella fusca* on growth, metabolism, and digestive functionality in juvenile thick-lipped grey mullet (*Chelon labrosus*). Fish were fed a control diet (CT) or a diet containing 15% *C. fusca* (C-15) biomass during 90 days. *C. labrosus* fed with the C-15 diet showed higher growth performance (in terms of final weight and length, weight gain, and specific growth rate) than the control group. Regarding fatty acids profile, *C. fusca*-fed fish showed a selective retention of DHA in the liver, and ARA, EPA, and DHA in the muscle. Dietary inclusion of this microalga significantly increased intestinal total alkaline protease, leucine aminopeptidase, and alkaline phosphatase activities in specimens fed with C-15 diet. Furthermore, intestine histological analysis revealed the absence of damage signs on gut morphology in fish fed the microalgae supplemented diet. Overall, the effects observed on digestive enzyme activities, together with adequate fatty acid profile, and gut morphology, and a significant increase in the intestinal mucosa's digestive and absorptive capacity, could explain the positive effects on growth performance obtained in fish fed the microalgae-supplemented diet. In conclusion, the results obtained showed that *C. fusca* is suitable as dietary ingredient for feeding thick-lipped grey mullet juveniles.