ORAL COMMUNICATIONS

BIOTECHNOLOGY

BT-C01

CHARACTERIZATION OF ANTARCTIC MICROBIAL PHOTOLYASES AND RECOMBINANT PRODUCTION

Marizcurrena JJ[1], Morales D[1], Martinez W[2], Castro Sowinski S[1]. [1]Bioquímica y Biología Molecular, Fac Ciencias, UdelaR. [2]Epigenetica e Inestabilidad Genomica, IIBCE. E-mail: j_jmarrena@hotmail.com.

Hymenobacter and *Sphingomona*s are microbial genera widely known for their high resistant to UV radiation. Photolyases are enzymes that repair the DNA lesions (cyclobutane pyrimidine dimers, CPD , and 6,4 photoproducts, 6,4-FP) produced by UV-irradiation (mainly by UVC). These enzymes are flavoproteins that belong to the photolyase/cryptochrome family. The aim of this work was the search for photolyases in two Antarctic UVC-resistant isolates, *Hymenobacter sp*. UV11 and *Sphingomonas sp*. UV9, and their photolyase in silico characterization. Both, UV11 and UV9 genomes were sequenced using Illumina HiSeq 2000 and annotated using RAST. The search in the annotated genomes showed that UV11 produce a CPD-photolyase, whereas UV9 produce two CPD-photolyases and a 6,4 photolyase. Their classification as novel CPD- or 6,4-photolyases was supported by their comparison with references sequences (by BLAST using NCBI and the cryptochrome DataBase) and by the construction of their evolutionary history. Their modeling and structure visualization/comparison were performed using HHpred and Pymol. The activity of the UV11 photolyase CDS was confirmed by recombinant production and activity using comet assays. Results suggest that UV9 and UV11 produce novel CPD- and 6,4-photolyases, with potential biotechnological applications.

BT-C02

BIOCHEMICAL CHARACTERIZATION OF A CELLULOLYTIC COCKTAIL FROM AN ANTARCTIC FLAVOBACTERIUM ISOLATE

Herrera Marrero LM[1], Braña V[1], Franco Fraguas L[2], Castro Sowinski S[1]. [1]Bioquímica y Biología Molecular, Fac Ciencias, UdelaR, Uruguay. [2]Bioquímica, Fac Química, Udelar. E-mail: herreramarrerolorena@gmail.com

Cellulases are a group of enzymes that hydrolyze the glycosidic bonds of cellulosic materials. They have many potential industrial applications, including pulp and paper and biofuel, among others. Thus, the search for novel cellulolytic enzymes that affect industrial strategies is continuously in progress. The aim of this work was the characterization of a cellulolytic raw preparation produced by a psychrophilic Antarctic isolate, *Flavobacterium sp*. AUG42. Psychrophilic microbes produce cold-adapted enzymes that usually show high catalytic efficiency, kcat/KM, as compared with enzymes produced by mesophilic microbes. AUG42 produces endo- and exo-cellulases at the stationary growth phase, using filter paper (FP), avicel, cellulose and carboxymethyl cellulose (CMC) as carbon source, showing the largest production using FP. The enzymatic preparation exhibited maximal endo- and exo-cellulase activity at 50°C/5.5 and 50°C/4.5 (temp/pH), respectively. In addition, CMC zymography and isoelectric focussing suggested the production of at least 4 cellulases with pI in the range of 4,5-7,5. Currently, the protein bands that showed activity are under analysis for MALDI-TOF MS. Both activities were inhibited by few compounds. These properties may allow their use as biocatalyst for saccharification of industrial wastes and fermentation by *S. cerevisiae* for the production of bioethanol.

BT-C03

CADMIUM AND LEAD RESISTANT RHIZOSPHERIC BACTERIA AS CANDIDATES FOR RHIZOREMEDIATION PROCESSESS

Saran A[1], Fernandez L[1], Massot F[2], Merini LJ[1]. [1]EEA-Anguil, INTA-CONICET. [2]Instituto NANOBIOTEC, UBA-CONICET. E-mail: saran.anabel@inta.gob.ar

Although *Helianthus petiolaris* has been identified as a cadmium and lead tolerant plant species, little is known about its rhizospheric microorganisms. Such microorganisms are crucial for enhancing the plant biomass production and tolerance to heavy metals, accelerating the phytoremediation process of contaminated soils. The aim of this work was to isolate and identify the cadmium and lead tolerant rhizospheric bacteria from *H. petiolaris* and assess its plant growth-promoting (PGPR) capabilities. Bacteria isolation was performed by washing the roots with saline and saline/Tween 80 solutions, which were inoculated into sterile GY media supplemented with either 10 mg $L^{-1}Cd^{+2}$ or 100 mg $L^{-1}Pb^{+2}$. After several rounds of enrichment culture in the GY/metal medium and subsequent purification by plate culture, nine different morphotypes were isolated. From biochemical analysis and 16S rRNA sequencing, the lead resistant bacteria were identified as *Bacillus subtilis, Klebsiella pneumoniae, Klebsiella oxytoca* and *Rhodococcus equi*. Identification of cadmium tolerant strains is in progress. In addition plant growth promotion capabilities, such as phosphate solubilization, phytohormones production (AIA), nitrogen fixation and siderophore production, were determined for the strains.

CELL BIOLOGY

CB-C01

NEURAL STEM CELL DIFFERENTIATION INDUCED BY LIPIDS

Montaner A; Costa M; Banchio C. Instituto de Biología Molecular y Celular de Rosario (IBR)- CONICET, Rosario, Argentina. E-mail: montaner@ibr-conicet.gov.ar

Neural stem cells (NSCs) have potential for self-renewal and differentiation into neurons, astrocytes and oligodendrocytes during nervous system development and after brain injuries; however, in the last case, this capacity is limited. We have previously shown that phosphatidylcholine (PtdCho) enhances neuronal differentiation while phosphatidyletanholamine (PtdEtn) promotes glia differentiation. By Time Lapse Microscopy analysis we concluded that these phospholipids affect precursors (undifferentiated post mitotic cells) but not progenitors (dividing cells) behavior. Furthermore, we demonstrated that these lipids neither affect the rate of cell proliferation nor the mode of NSCs division. By immunofluorescence analysis using specific markers, we evidenced that PtdCho acts on unspecified precursors driving neuronal specification and, as a consequence, promoting neurogenesis. Furthermore, under this condition, astrogenesis is reduced due to a decrease in astrocytes precursors. In the case of PtdEtn, it enhances astrocytes specification and differentiation. To get insight into the molecular mechanism, we analyzed the signaling pathways that participate in each differentiation process specifically altered by PtdCho or PtdEtn, and identified that PtdEtn but not PtdCho induces astrocyte differentiation activating the MEK-ERK pathway.

CB-C02

SUPPRESSION OF STARD7 PROMOTES ENDOPLASMIC RETICULUM STRESS AND INDUCES ROS PRODUCTION

Flores Martín J; Reyna L; Ridano ME; Panzetta-Dutari GM; Genti-Raimondi S. Dpto. Bioquímica Clínica, Facultad de Ciencias Químicas-UNC. CIBICI-CONICET. Argentina. E-mail: jflores@fcq.unc.edu.ar.

StarD7 transcript encodes an intracellular lipid transport protein, a member of the START domain superfamily, which is involved in many physiological processes. It facilitates the delivery of phosphatidylcholine to the mitochondria and previous results indicated that StarD7 knockdown decreases A*CB*G2 multidrug transporter level, cell migration, proliferation, and phospholipid synthesis. Since lipids and protein transport between organelles are involved in the organization of the different cell compartments, we hypothesized that StarD7 may be involved in maintaining cell homeostasis. We analyzed the effect of StarD7 silencing on endoplasmic reticulum (ER) stress response and on the production of reactive oxygen species (ROS) in HepG2 cell line. StarD7 knockdown generated alterations in mitochondria and ER morphology, initiating an unfolded protein response pathway associated with an increased basal ROS and augmented levels of the hemeoxygenase-1 and catalase enzymes. Also, StarD7 silencing reduced cell viability after H2O2 exposure. Moreover, downregulation of p53 by a degradation mechanism was established in StarD7 siRNA cells. Finally, no changes in autophagy and apoptosis were observed in StarD7 siRNA. Together these results indicate that, beyond its role in lipid transport, StarD7 contributes to maintain cellular redox homeostasis.

CB-C03

STRATEGY TO STUDY PARAMETERS ABLE TO PREDICT LONGEVITY IN MEDFLY POPULATIONS

Bocchicchio P[12]; Pujol-Lereis L[1]; Rossi F1; Turdera L[2]; Pérez M[13]; Rabossi A[13]; Quesada-Allué L[12]
[1].IIBBA-CONICET [2].Depto.Quim.Biol. FCEyN-UBA. E-mail: pbocchicchio@leloir.org.ar.

The Medfly (*C.capitata*) is the main orchards world pest. Functional Senescence refers to real physiological output, eventually leading to different mortality rate. To predict changes in longevity dependent on genetic microheterogeneity of Medfly lab populations subjected or not to stress we worked out both lethal and non-lethal assays. To establish a parameter reflecting different homeostatic equilibria, we analyzed quantitative changes of 38 Lipids categories, further reduced to significant 25 ones. Thus giving rise to Principal Components for each sex and body parts. Higher temperature of growing, chill coma induction or oxidative stress induced by Hematoporphyrin were the main factors studied. These and other biochemical and gene expression parameters were correlated with non lethal estimations of behavioral output like mobility and RING (negative geotaxis) experiments. Looking further for longevity predictors we analyzed spontaneous and/or fight-induced supine events during the early adulthood of medflies. We demonstrated a correlation between the rate of supine events and longevity that also roughly correlated with changes in biochemical and gene expression parameters. Most important, supine rate also correlated with other non-lethal assays like RING. Age- and stress-dependent changes in a peculiar immune reaction were also explored

CB-C04

LOW NRF2 EXPRESSION DETERMINES LOW ADIPOGENESIS, INFLAMMATION AND HIGH METABOLIC RISK IN BOYS AND RA

Santillan LD, Gimenez MS, Ramirez DC. [1]Lab. of Exp. and Transl. Med. & [2]Lab. Nutr. and Environ. IMIBIO-SL-CONICET-UNSL. E-mail: lucassantillan2011@gmail.com.

Excess of energy is metabolized to free fatty acids which should be stored as triglycerides otherwise they cause inflammation. Nrf2 controls the expression of phase II, antioxidant and adipogenic genes. Low Nrf2 expression may determine inflammation and a high metabolic risk in obesity. To test this hypothesis we made a study in children and in an experimental model in rats. We measured clinical and biochemical parameters related to lipid metabolism, oxidative stress and metabolic syndrome in a population of overweight boys (OW, n=22) and normal weight boys (NW, n=27) from San Luis City. Compared to NW, OW boys had insulin resistance, higher atherogenic index, altered plasma lipid profile, increased markers of oxidative stress and inflammatory fatty acids. GPx activity and GSH/GSSG ratio and

leukocyte Nrf2 expression were also lower. Nrf-2 expression negatively correlated with metabolic syndrome parameters in OW boys. Experimentally we fed SD rats (n=12) for 16 weeks with a hypercaloric diet and found that some rats were obesity sensitive (OS, n=7) whereas the others were obesity resistant (OR, n=5). Compared to OS and in perirenal adipose tissue, OR rats showed increased oxidative stress (NOX2), inflammation (VCAM), but reduced Nrf2, PPAR-γ and lipogenic enzyme expression. Low Nrf2 expression determines reduced adipogenesis; but increased inflammation and metabolic risk.

CB-C05

NATURAL GENETIC VARIATION DETERMINES PROMOTER SHAPE, AFFECTING ROBUSTNESS OF GENE EXPRESSION

Schor I[12]; Degner JF[2]; Harnett D[2]; Cannavo E[2]; Casale FP[3]; Garfield D[2]; Stegle O[3]; Furlong EE[2]. [1]IFIBYNE (CONICET)-DFBMC (FCEN, UBA). [2]GB Unit, EMBL-Heidelberg (Germany); [3]EMBL-EBI (UK). E-mail: ieschor@fbmc.fcen.uba.ar.

Promoters act as platforms for converging cis-regulatory cues, which regulate the initiation of gene expression. Metazoan core promoters have characteristic distributions of transcriptional start sites (TSS), a feature called promoter shape. Although fundamental to understanding transcriptional initiation, the genetic determinants and functional differences between promoters with different shapes remain unclear. Here, we measured TSS usage across a panel of 81 *Drosophila* lines, identifying thousands of thousands of promoters where promoter strength and/or shape are modulated by common genetic variants. Our results identify promoter shape as a variable trait, evolvable independently of promoter strength. Broad promoters, typical of housekeeping genes, show an increase in the number of genetic variants affecting transcription. Since these variants have lower impact in promoter strength than those of narrow promoters, broad promoters appear as intrinsically more robust to genetic variation. By using single-cell expression analysis, we show that shape-shift mutations frequently increase expression noise, while other variants within the same promoter can alleviate these effects, often through epistatic interactions. This study uncovers new functional properties of natural promoters, and proposes the minimization of noise as an important factor influencing promoter evolution

CB-C06

MITOCHONDRIA-TARGETED CATALASE PREVENTS OXIDATIVE STRESS AND REVERTS ANTIOXIDANT RESPONSE IN DOWN SY

Helguera PR; Zamponi E; Busciglio J. Instituto de Investigación Médica Mercedes y Martín Ferreyra. E-mail: prhelguera@immf.uncor.edu.

The free radical theory of aging proposes that cumulative damage caused by reactive oxygen species ROS is responsible of the functional decline of living systems, leading to diseases and eventually death. A successful longevity murine model was designed based on these concepts, targeting over expression peroxide degrading enzyme catalase to mitochondria (mCAT), the main center of reactive oxygen species (ROS) production. Our group has been studying aging in Down syndrome (DS). We have described for DS cells chronic oxidative stress associated with mitochondrial dysfunction that ends up compromising cellular functions, associated to transcriptome showing active antioxidant systems Nrf2 pathway, a master regulator of antioxidant response elements (ARE). Here we are presenting the characterization of mCAT expression in DS cells, using a lentiviral system. The strategy was efficient decreasing mitochondrial chronic oxidative damage, restoring mitochondrial membrane potential and recovering the organelle's structure in these cells. We observed that mitochondrial dysfunction affected DS fibroblasts migration capacity and that mCAT rescued this parameter, too. Finally we explored Nrf2 pathway activation in DS fibroblasts, confirmed its upstream regulators, and observed that mCAT activity impacted the cellular stress response diminishing Nrf2 stabilization and nuclear translocation.

CB-C07

MAPPING THE DYNAMICS OF THE GLUCOCORTICOID RECEPTOR AND ITS COREGULATOR NCOA-2 IN THE NUCLEUS

Stortz MD[1] , Presman DM[2], Bruno L, Annibale P[4], Hager GL[2], Gratton E[4], Levi V[56], Pecci A[15]. [1]IFIBYNE-CONICET. [2]NIH, USA. [3]IFIBA-CONICET [4]LFD, UC Irvine, USA. [5]QB, FCEN-UBA. [6]IQUIBICEN-CONICET. E-mail: mstortz@qb.fcen.uba.ar.

The distribution of the transcription machinery among different subnuclear domains raises the question on how this organization modulates the transcriptional response. We used imaging and fluorescence correlation spectroscopy (FCS) based approaches to quantitatively explore the dynamical intranuclear organization of the glucocorticoid receptor (GR) in living cells. This ligand-activated transcription factor diffuses within the nucleus and can engage in shorter and longer-lived interactions with chromatin targets or interact with DNA-dependent foci or promyelocytic leukemia (PML) bodies, which accumulate the coregulator NCoA-2. We also analyzed the recruitment and binding properties of GR and its coregulator to glucocorticoid response elements (GREs). The distribution of the receptor among these different nuclear compartments depends on the interaction with NCoA-2 and GR conformation, as assessed with synthetic ligands and receptor mutants with impaired transcriptional abilities. Our results suggest that the partition of the GR in different nuclear reservoirs may represent another step of transcription regulation, since the interaction with these compartments could ultimately influence the ability to bind to the specific transcriptional targets

CB-C08

ANTITUMORAL EFFECTS OF BIOENERGETIC MODULATION IN FELINE MAMMARY CARCINOMA CELLS

Arbe MF[1], Fondello C[1], Agnetti L[1], Tellado M[2], Alvarez G[2], Glikin GC[1], Finocchiaro LM[1], Villaverde MS[1]. [1]UTG, Área de Investigación, IOARH, FMed, UBA. [2]Cátedra de Química Biológica, FVet, UBA. E-mail: florenciaarbe@hotmail.com.

Feline mammary carcinoma (FMC) is a highly aggressive pathology that has been proposed as an interesting model of breast cancer disease. Metabolism has been described as a hallmark of cancer cell. The aim of the present work was to investigate the effects and mechanism of the modulation of metabolism by metformin (MET, an oral anti-diabetic drug), 2-deoxyglucose (2DG, HK inhibitor) or a combination of both drugs, MET/2DG on two established FMC cells lines: AlRB (HER2 (3+) and Ki67<5%) and AlRATN (HER2 (-) and Ki67>15%). Treatments decreased both FMC cells viability in a concentration dependent manner (APH assay, $p<0.05$). AlRB was more sensitive to 2DG than AlRATN (IC50: 3.15 vs 6.32 mM, respectively). The combination of MET/2DG potentiated the individual drugs cytotoxic effects on FMC cells. Only MET/2DG caused an increased in intracellular oxidants (as determined by DCF, $p<0.05$), AO positive vesicles (related to autophagic mechanism, flow cytometry, $p<0.05$) and completely inhibit colony formation. On the contrary only MET significantly altered plasma membrane integrity (by increasing PI uptake, $p<0.05$), presented late apoptotic/necrotic cells (AO/EB staining, $p<0.05$) and increased both glucose consumption and lactate concentration. This results support further studies to investigate the potential use of this metabolic modulation approach in a clinical veterinary setting.

ENZYMOLOGY

EN-C01

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL STARCH BRANCHING ENZYME FROM *Ostreococcus tauri*

Hedin N, Barchiesi J, Gomez-Casati DF, Busi MV. CEFOBI-CONICET-UNR. Facultad de Ciencias Bioquímicas y Farmacéuticas. Rosario, Argentina. E-mail: hedin@cefobi-conicet.gov.ar

Starch branching enzyme (BE) is a highly conserved protein from plants to algae. This enzyme participates on starch granule assembly by the addition of α-1,6 glucan branches in the α-1,4 polyglucans. This modification determines the fine structure of amylopectin, and thus, the final structure of the starch granule. In this work we describe the function of a starch branching enzyme from the picoalgae *Ostreococcus tauri*. Although previous *in silico* evidence suggested that this protein is a starch debranching enzyme, structure-function studies confirmed that this polypeptide is a BE comprising two in tandem carbohydrate binding domains (from CBM41 and CBM48 families) at the N-terminal end of the protein followed by a C-terminal catalytic domain. The analysis of truncated isoforms shows that the CBMs bind differentially to starch and the distinct starch fractions. Moreover, no catalytic activity was detected in the CD alone or with the truncated forms of the protein. The results suggest that this *O. tauri* protein is a functional BE containing a CBM41 and CBM48 that are essential for enzyme activity and regulation.

EN-C02

KINETIC AND FUNCTIONAL CHARACTERIZATION OF OTDSP, A PHOSPHOGLUCAN PHOSPHATASE FROM *O. tauri*

Carrillo JB, Martín M, GomezCasati DF, Busi MV. CEFOBI-CONICET. Facultad de Ciencias Bioquímicas y Farmacéuticas. Suipacha 531. Rosario, Argentina. E-mail: carrillo@cefobi-conicet.gov.ar

Over the past decade, the phosphoglucan phosphatases have surfaced as fundamental proteins that regulate storage carbohydrate metabolism in plants as well as mammals. The functional mechanism of these enzymes is understood but little is known about its evolution in the green lineage and no reports exist concerning green algae. In this sense, we have identified and characterized a novel glucan phosphatase of the ancient picoalga *Ostreococcus tauri*. We verified OtDSP phosphatase activity *in vitro* with pNPP as well as its natural substrate amylopectin, but with a different kinetic behavior. To further characterize the enzyme and based in OtDSP homology with *A. thaliana* LSF2 we identified amino acidic residues involved in catalysis and binding to substrate that were mutagenized. Wild type OtDSP and its mutants counterparts were studied by means of native-PAGE, size exclusion chromatography and binding assays to polysaccharides. The results obtained suggest OtDSP as a fully functional enzyme *in vivo*.

EN-C03

ALTERNATIVE CATALYTIC PROPERTIES IN THE GLYCOGEN-SYNTHASE FROM ACTINOBACTERIA

Asencion Diez MD[1], Cereijo AE[1], Alvarez HM[2], Iglesias AA[1]. [1]IAL (UNL-CONICET) [2] Instituto de Biociencias de la Patagonia (UNPSJB-CONICET. E-mail: masencion@fbcb.unl.edu.ar

A new pathway (named GlgE) for prokaryotic glycogen metabolism was described in Actinobacteria. In this pathway, the key enzyme GlgE extends the glucan in two glucose units by means of maltose-1P; whilst in the classical GlgAC pathway glycogen-synthase (GlgA, EC 2.4.1.21) elongates the polymer in one unit, using ADP-glucose as the glucosyl donor. Recently it was reported that the mycobacterial GlgA catalyzes maltose-1P synthesis consuming glucose-1P and ADP-glucose as substrates. Thus, we analyzed this reaction in several GlgAs from different sources so far characterized in our lab. Amongst them, GlgAs from Actinobacteria (Streptomyces and Rhodococcus) were active for maltose-1P synthesis. Moreover, both actinobacterial GlgA showed some degree of promiscuity towards sugar-1Ps but not for NDP-sugars. Particularly, rhodococcal GlgA used glucosamine-1P to the same extent than glucose-1P. Actinobacterial GlgAs also catalyzed the synthetic reaction using ADP-Glc and maltose-1P (~50% lower regarding glucose-1P) as substrates. In addition, the maltose-1P forming activity was detected in crude extracts from *Rhodococcus jostii*, thus suggesting a biological significance for this alternative catalytic property. Results support a critical role of maltose-1P and a multifaceted GlgA function (being involved in the classical as well as in the GlgE pathway) for glycogen metabolism in actinobacteria.

LIPIDS

LI-C01

HIGH-NACL INDUCES SREBP-MEDIATED TRANSCRIPTIONAL REGULATION OF TRIGLYCERIDES

Weber K; Casali CI; Malvicini R; Parra LG; Etcheverry T; Fernandez MC UBA, FFYB, BCM; CONICET-IQUIFIB E-mail: kweber@ffyb.uba.ar

Hypertonicity regulates phospholipids (PLs) and TAG synthesis. These metabolic pathways can be regulated by transcriptional activation of their biosynthetic enzymes. Several transcription factors may be involved in such regulation but sterol response element binding protein (SREBP) is considered the master regulator of lipogenic genes. We showed that MDCK cells subjected to high NaCl induce changes in mRNA expression and cell distribution of SREBP isoforms. These changes were consistent with the increased levels of PLs and TAG in treated cells and with the decrease in lipid synthesis after fatostatin treatment. However, we did not establish which isoform, SREBP1 (S1) and/or SREBP2 (S2), is responsible for the increased lipogenic activity. The present work was aimed to address this. Before the addition of hypertonic medium, MDCK cells were treated with S1-siRNA, S2-siRNA or both. After NaCl treatment lipid synthesis was studied. PLs and 1,2 DAG synthesis were not affected by any siRNA. In contrast, both 1,3 DAG and TAG synthesis were blocked. S1-siRNA decreased DAG and TAG synthesis by 33 and 46 %. S2-siRNA decreased DAG and TAG by 40 and 37%, respectively. Both siRNAs reduced synthesis by 55 %. So, SREBPs are needed to maintain TAG synthesis and its degradation to DAG but PLs synthesis remains constant indicating that SREBP-mediated transcriptional regulation is not involved

LI-C02

THE ETHER-LINKED LIPIDS OF RAT EPIDIDYMIS ARE AFFECTED BY MILD HYPERTHERMIA

Luquez JM; Santiago Valtierra FX; Oresti GM; Aveldaño MI; Furland NE INIBIBB, CONICET-UNS, 8000 Bahía Blanca, Argentina E-mail: jluquez@criba.edu.ar

It is widely known that heat stress temporarily suppresses spermatogenesis in the mammalian testis, but the impact on the epididymis is barely known. The aim of this study was to examine the effects of short, repeated once-a-day episodes of hyperthermia (43ºC) on the ether-linked glycerophospholipids (GPL) and triglycerides (TG) of rat epididymis. One-week post-treatment, the expression (mRNA) of alkylglycerone phosphate synthase (AGPS), a key peroxisomal enzyme in the synthesis of these lipids, significantly fell in caput and corpus epididymis. Coincidentally, levels of the plasmalogen precursor, plasmanyletanolamine, decreased. Concurrently, plasmenylethanolamine and plasmenylcholine accumulated in caput, ascribable to injured and motionless cells and sperm collecting in the lumen. Catabolism of such GPL had started in the epididymal epithelium, as suggested by the build-up of ether-linked TG. Between weeks 2 and 6, spermatogenesis restarted in the testis. Although the epididymis was still sperm-free at week 6, its levels of ether-linked GPL and TG were much higher than those of untreated controls, in agreement with the recovery of AGPS expression. Ether-linked TG were formed by de novo synthesis and during GPL breakdown. The presence of spermatozoa in the epididymal lumen apparently plays a regulatory role in the biosynthesis of ether-linked lipids by the epididymal epithelium

LI-C03

ROLE OF GPA3/4 IN GLYCEROLIPID SYNTHESIS, PHAGOCYTOSIS AND CYTOKINE RELEASE IN ACTIVATED MACROPHAGES

Quiroga IY[1]; Pellon-Maison M[1]; Coleman RA[2]; Gonzalez-Baro MR[1] [1]INIBIOLP-UNLP-La Plata, Argentina [2]Dept. Nutrition, UNC, USA E-mail: yoseli_quiroga@hotmail.com

Glycerol-3-phosphate acyltransferase (GPAT) regulates de novo glycerolipid synthesis. GPAT activity is up-regulated during macrophage activation, when PL and TAG accumulation in lipid droplets (LDs) is increased. We studied the role of GPAT3 and GPAT4 during macrophage activation in a shGpat3 macrophage cell line and Gpat3$^{-/-}$and Gpat4$^{-/-}$ mice Bone Marrow Derived Macrophages (BMDM). All the LPS-activated Gpat-silenced macrophages accumulate less LDs, TAG and PL than the Gpat-expressing control cells. We analyzed the incorporation of [^{14}C]-Acetate and [^{14}C]-Oleic acid (OA) into lipids in activated shGpat3 cells, Gpat3$^{-/-}$ and Gpat4$^{-/-}$ BMDM; the incorporation of both substrates decreased in the absence of GPAT3 or 4 and while GPAT3 participates in both PL and TAG synthesis, GPAT4 is mostly involved in TAG synthesis. To investigate the physiological effect of impaired lipid synthesis, we analyzed the phagocytic capacity of shGpat3 cells, Gpat3$^{-/-}$and Gpat4$^{-/-}$ BMDM and it was 45, 22 and 31% lower than in the activated controls. We found that the expression and cytokine release during macrophage activation in these cells was also altered. Taken together, these results prove that GPAT3 and 4 contribute to the increase in total glycerolipid content, phagocytosis and cytokine production during macrophage activation.

LI-C04

A METABOLIC CIRCADIAN CLOCK CONTROLS RHYTHMS IN IMMORTALIZED HUMAN GLIOBLASTOMA T98G CELLS

Wagner PM[1]; Sosa-Alderete L[1]; Gorné L[1]; Gaveglio V[2]; Salvador G[2]; Pasquare S[2]; Guido ME[1]

[1]CIQUIBIC-CONICET, Dept. Biol Chem. FCQ-UNC, Cordoba, Argentina. [2]INIBIBB-CONICET. Bahía Blanca. E-mail: pwagner@fcq.unc.edu.ar

Circadian clocks present even in immortalized cell lines temporarily regulate diverse physiological processes and can be synchronized by different ambient signals. The disruption of circadian rhythms may lead to metabolic disorders or higher cancer risk through failures in cell division control. Previous results in immortalized human glioblastoma T98G cells showed that clock genes (*Bmal1*, *Per1*, *Rev-Erbα*), some phospholipid (PL) synthesizing enzyme genes and the labelling of ^{32}P-PLs exhibited different temporal profiles depending on the growth condition tested (proliferation: P, partial arrest: A) with metabolic rhythms mainly preserved under P. Here we evaluated redox metabolism (redox state and peroxiredoxin oxidation cycles) and the activities of PL synthesizing enzymes for phosphatidate phosphohydrolase (PAP) and lysophospholipid acyl transferases (LPLAT) in T98G cells under P or A, synchronized with dexamethasone (100 nM) (time 0) and collected at different times for 36 h. Results showed that redox state, peroxiredoxin oxidation cycles and PAP activity exhibited temporal oscillations in both growth conditions tested (P and A) while LPLAT activity seems to be rhythmic under P. Our observations support the idea that a metabolic clock could operate in these tumor cells regardless the molecular clock which was not found to work properly under proliferation.

LI-C05

EXPRESSION OF ELOVL4 AND FA2H WITH SPERMATOGENIC CELL DIFFERENTIATION IN THE RAT TESTIS

Santiago Valtierra FX; Peñalva DA; Luquez JM; Furland NE; Aveldaño MI; Oresti GM INIBIBB, CONICET-UNS, Bahía Blanca, Argentina E-mail: gmoresti@criba.edu.ar

Rat spermatogenic cell membranes contain sphingolipids with nonhydroxy and 2-hydroxy very long chain (C_{24-32}) PUFA. The biosynthesis of such fatty acids requires the expression of very long chain fatty acid elongases (*Elovl4* for > C_{24}) and a fatty acid 2-hydroxylase (*Fa2h*). In this study, mRNA levels of *Elovl4*and *Fa2h* were measured by qPCR in rat testis at different postnatal ages and in cells isolated from the seminiferous epithelium of adults. At early prepuberal ages (P14), *Elovl4* was highly expressed while *Fa2h* mRNA was absent. *Fa2h* started to be detected at P25-30 and increased thereafter, while *Elovl4*mRNA levels decreased. The expression of both genes, but mainly *Fa2h*, was markedly reduced in adult testes that had been depleted of germ cells by mild hyperthermia. In isolated spermatogenic cells, both genes were expressed at lower levels in pachytene spermatocytes than in post-meiotic round and late spermatids. Interestingly, Sertoli cells had high *Elovl4*but lacked *Fa2h* mRNA. The Elovl4 protein was detected in spermatocytes from P21 to adulthood, when the protein was clearly observed in elongated spermatids. The Elovl4 enzyme was functional in germ cells, as these cells, in culture, were able to elongate [^{3}H]20:4 to PUFA longer than C_{24}. Our results underscore the presence of a well-timed, cell-specific regulation of *Elovl4* and *Fa2h* in germ cells as differentiation proceeds.

LI-C06

LOW-DENSITY MEMBRANE FRACTIONS FROM MALE GERM CELLS LACK SPHINGOLIPIDS WITH VERY LONG CHAIN PUFA

Santiago Valtierra FX; Mateos MV; Aveldaño MI; Oresti GM INIBIBB, CONICET-UNS, Bahía Blanca, Argentina E-mail: gmoresti@criba.edu.ar

Sphingomyelins (SM) and ceramides (Cer) with very long-chain PUFA (VLCPUFA), in nonhydroxy (n-V) and 2-hydroxy (h-V) forms, are specific components of rat spermatogenic cells. Here we evaluated how differentiation affects their distribution among membrane fractions from such cells. Using a detergent-free procedure, a small light, raft-like low-density (L) fraction and a large heavier (H) fraction, both showing markers typical of cell plasma membranes, were separated from pachytene spermatocytes, round, and late spermatids. MALDI-TOF spectra showed that the L fraction had mostly SM species with saturated fatty acids regardless of the cell stage, while the H fraction was rich in stage-varying SM and Cer species with VLCPUFA. In this fraction spermatocytes accumulated mostly n-V SM and spermatids h-V SM and h-V Cer species. A third fraction made of intracellular membranes had less SM and more Cer than the H

fraction, differentiation also increasing the h-V/n-V ratio in both lipids. The buildup of 2-hydroxy fatty acids correlated with the expression (mRNA) of fatty acid 2-hydroxylase (Fa2h), higher in spermatids than in spermatocytes. The differentiation-dependent rise in h-V Cer in the germ cell H fraction during spermatogenesis is consistent with the eventually uneven distribution that n-V and h-V species of SM and Cer display between the head and the tail of mature spermatozoa.

MICROBIOLOGY

MI-C01

THE ROLE OF RESPIRATORY OXIDASES IN THE MECHANISM OF ACTION OF MICROCIN J25

Galván AE[1], Chalón MC[1], Schurig-Briccio L[2], Minahk CJ[1], Gennis R[2], Bellomio A[1]. [1]INSIBIO (CONICET-UNT). Tucumán, Argentina. [2]Deparment of Biochemistry. University of Illinois. E-mail: emilcegalvan@hotmail.com

The antibacterial peptide microcin J25 (MccJ25) displays an antibiotic activity against *Salmonella*, *Shigella* and *Escherichia coli*. MccJ25 has two cellular targets, the RNA polymerase and the respiratory chain. The terminal oxido-reductases in *E. coli* respiratory system are the cytochromes bo_3 and *bd*. We studied the effect of MccJ25 in *E. coli* C43 cytochrome mutant strains. The oxygen consumption was diminished by MccJ25 in the wild type strain and in the mutant strain lacking the cytochrome bo_3, but did not have any effect in Δ*bd*I strain. In the same way, superoxide production in isolated membranes was increased more than 100 % in the control and Δbo_3 strain, whereas in cytochrome *bd* mutant such increment was not observed. Moreover, working with purified cytochromes, MccJ25 inhibited about 25 % the ubiquinol oxidase activity only on cytochrome *bd*I, while under identical experimental conditions bo_3 oxidase was insensitive to the peptide. These results demonstrate that cytochrome *bd*I plays an important role in the microcin J25 mechanism of action on the respiratory chain of *E. coli*. Our findings would provide a new insight into the application of MccJ25 in food or pharmaceutical industries.

MI-C02

FUNCTIONAL CHARACTERIZATION OF THE CELL DIVISION PROTEIN FtsA OF *Streptococcus pneumoniae*

Yandar NY, Reinoso N, Cortes PR, Echenique J. Dpto. Bioquimica Clinica/CIBICI-CONICET, Fac. Cs. Químicas, UNC. E-mail: nyandar@fcq.unc.edu.ar

FtsA is a divisome protein that connects the master coordinator of cell division, FtsZ, to the cell membrane for tethering the Z-ring and septal formation. Previous reports have showed that FtsA forms a ring-like structure at the division site of streptococcal cells. In addition, some authors reported that *ftsA* is an essential gene. In this work, we could obtain the *ftsA* mutant by insertion mutagenesis demonstrating that *ftsA* is dispensable for cell viability. However, the *ftsA* mutant displayed fitness and morphological alterations. By fluorescence microscopy, we also found a delocalization of FtsZ-GFP in the *ftsA* mutant, phenotype that is compatible with the known FtsA function. The wild-type shape, cell cycle and FtsZ localization were recovered when the *ftsA* cells were complemented by expression of *gfp-ftsA*. By confocal microscopy, we detected the reported localization of GFP-FtsA at the midcell in the wild-type strain, but we also observed an unexpected localization during cell cycle progression. This pattern was confirmed by expression of FtsA fused to HA (human influenza hemagglutinin tag) and revealed with an anti-HA monoclonal antibody. These results revealed new features of FtsA and confirmed that it is an essential piece of the cell division mechanism of *S. pneumoniae*.

MI-C03

REGULATION OF THE SUBPOLAR FLAGELLUM SYNTHESIS IN *Bradyrhizobium diazoefficiens*

Dardis C, Mengucci F, Althabegoiti MJ, Lodeiro AR, Quelas JI, Mongiardini EJ. Instituto de Biotecnología y Biología Molecular (IBBM) CCT-La Plata CONICET, UNLP. E-mail: carolinadardis@biol.unlp.edu.ar

Bradyrhizobium diazoefficiens is the soybean nitrogen-fixing symbiont commonly used in inoculant formulations. This α-proteobacterium uses two independent flagellar systems to swim in liquid and viscous media. In our laboratory, we studied the synthesis and regulation of both flagellar systems, and here we show part of the regulatory cascade of the subpolar flagellum synthesis. Flagellar synthesis occurs in steps, each one controlled by different regulators. This process ensures the appropriate timing of the synthesis of different components. First, a master regulator initiates the signal cascade, then class II regulators control gene expression of the intermediate products and class III/IV regulators activate flagellum filament formation, the last product assembled. We present the characterization of *B. diazoefficiens* mutants in two class II regulatory genes (*flbD* and *fliX*) and two class III regulatory genes (*flaF* and *flbT*), by measuring the

transcription levels of the putative targets controlled by them and also the type of flagellins that they produced. Our results suggest that the regulation of the subpolar flagellum synthesis is independent from the lateral flagella and is controlled in a cell-cycle manner. These results fit with the model previously described in *Caulobacter crescentus* but not with *Salmonella* model, as was thought in earlier studies.

MI-C04

ENTEROBACTIN: A FENTON-SAFE SIDEROPHORE

Peralta DR, Adler C, Corbalán NS, Paz García EC, Pomares MF, Vincent PA. INSIBIO, CONICET-UNT. Chacabuco 461, T4000ILI – Tucumán, Argentina. E-mail: drperalta@fbqf.unt.edu.ar

There is increasing evidence that siderophores may play alternative roles, aside of providing cells with the necessary iron. We previously reported that the catechol siderophore enterobactin, is crucial for *Escherichia coli* colony development in culture conditions that increased the oxidative stress. In this work, we demonstrated that enterobactin confers protection against various sources of oxidative stress such us H_2O_2, paraquat and copper, independently of its ability to facilitate iron uptake. Protection against oxidative stress occurs in the cell cytoplasm through ROS scavenging and requires prior hydrolysis of the enterobactin molecule. Interestingly, both the sensitivity to stressors and the colony development arrest phenotype were enhanced when cells harbored the *entE* mutation along with either *sodA* or *katG*. Confirming the link between enterobactin and oxidative stress, we found that enterobactin transcriptional expression and production was induced by oxidative stressors even in the presence of iron. Furthermore, preliminary data indicates that enterobactin transcription would be regulated by oxidative stress through the global regulator SoxS. These results strongly support the involvement of enterobactin as part of the oxidative stress response of *E. coli*.

MI-C05

THE *map* LOCUS OF *Brucella suis* IS INVOLVED IN CELL ENVELOPE BIOGENESIS AND VIRULENCE

Bialer MG[1], Ruiz-Ranwez V[1], Estein SM[2], Russo DM[1], Altabe SG[3], Sycz G[1], Zorreguieta A[1]. [1]Fundación Instituto Leloir, IIBBA-CONICET, Bs.As. [2]CIVETAN-CONICET, Tandil. [3]IBR-CONICET, Rosario. E-mail: mbialer@leloir.org.ar

Brucella species exhibit unique surface properties, which make them furtive pathogens and more resistant to several host defense compounds. We have identified a *locus* of *Brucella suis* encoding the MapA and MapB proteins, which are predicted to represent the TAM machinery, recently proposed to participate in the translocation/insertion of autotransporters (ATs) in the outer membrane (OM). In *Brucella*, ATs are involved in bacterial attachment to host components. However, the role of TAM in *B. suis* would not be restricted to AT translocation since the Δ*mapB* phenotypes were not only related to adhesion functions. Indeed, Δ*mapB* showed enhanced sensitivity to lysozyme, Triton X-100 and polymyxin B, indicating that the cell envelope integrity is compromised. This effect was not due to major differences in the LPS structure or to altered total fatty acid composition. Analysis by LC-MS/MS and *Western Blot* of membrane fractions suggested that the extent of some OM proteins is slightly altered in the *mapB* mutant. Interestingly, the number of bacteria recovered from macrophages during the initial stages of infection was reduced in the mutant and it showed an attenuated phenotype in mice. These results suggest that MapA/MapB assists in the correct insertion of an unknown subset of protein substrates or other OM components, which are important for OM stability and virulence.

MI-C06

CLONING, EXPRESSION & CHARACTERISATION OF THE HEPATITIS E VIRUS CAPSID PROTEIN OF GENOTYPES 1-4 FOR SERODIAGNOSTIC

Arce L[1], Stellberger T[2], Baiker A[2], Vizoso Pinto MG[1]. [1]INSIBIO (UNT-CONICET). Facultad de Medicina de la Univ. Nac. De Tucumán. Argentina. [2]LGL, Erlangen, Germany

The National Institutes of Health classified Hepatitis E as an emerging disease because: Hepatitis E Virus (HEV) is the major cause of acute hepatitis in developing countries, there is an increasing number of HEV infections in industrialized countries associated to live stock and, chronic HEV infections are being reported in immunocompromised patients (HIV, transplant recipients, etc). HEV transmission by blood transfusions has also been recently reported. There are scarce epidemiological data about HEV occurrence and prevalence in Argentina, and diagnostic tests are needed. Open reading frame 2 (ORF2) of HEV genotypes (GT) 1 to 4 were recombinatorially cloned into a customized, N-terminally His-tagged bacterial expression vector and analysed for recombinant protein expression and purification. We optimized expression under native and denaturing conditions. Purified proteins were blotted onto a nitrocellulose membrane (dot-blot) and examined for their antigenic potential by serum profiling experiments. Although the four established GTs of HEV belong to a single serotype, the serum of a patient infected with HEV ORF2 GT3 reacted with more intensity with ORF2 GT3. We provide a prototype of immunoassay for complementary confirmation of HEV seropositivity as detected in screening assays such as ELISA and plan to test a panel of sera from Argentinean blood donors.

NEUROSCIENCE

NS-C01

THE VISUAL CYCLE IN THE INNER RETINA OF CHICKEN AND THE ROLE OF RETINAL G-PROTEIN-COUPLED RECEPTOR

Diaz NM[1]; Morera LP[1]; Tempesti TC[2]; GuidoME[1] [1] CIQUIBIC-CONICET, FCQ, UNC [2] INFIQC-CONICET, FCQ UNC, Cordoba Argentina E-mail: mguido@fcq.unc.edu.ar

The vertebrate retina contains typical photoreceptor (PR) cones and rods responsible for vision and intrinsically photosensitive retinal ganglion cells (ipRGCs) involved in the regulation of non-visual tasks. Visual photopigments in visual PRs or melanopsin (Opn4) in ipRGCs utilize retinaldehyde as a chromophore. The retinoid regeneration process denominated as "visual cycle" involves the retinal pigment epithelium (RPE) or Müller glial cells. However, it is unknown how the chromophore is further metabolized in the inner retina. Nor is it yet clear whether an alternative secondary cycle occurs involving players such as the retinal G-protein-coupled receptor (RGR), an opsin of unidentified inner retinal activity. Here, we investigated the role of RGR in retinoid photoisomerization in Opn4x (+) RGC primary cultures from chicken embryonic retinas. Opn4x (+) RGCs display significant photic responses and photoisomerize exogenous all-trans to 11-cis Ral and other retinoids. RGR was found to be expressed in developing retina and in primary cultures; when its expression was knocked down, the levels of retinals and retinol in cultures exposed to light were significantly higher and those in all-trans retinyl esters lower than in dark controls. The results support a novel role for RGR in ipRGCs to modulate retinaldehyde levels in light, keeping the balance of inner retinal retinoid pools.

NS-C02

METABOLIC DYSFUNCTION WORSENS COGNITION AND NEURONAL RESILIENCE IN A RAT MODEL OF EARLY ALZHEIMER

Martino Adami PV[1]; Galeano P[1]; Wallinger ML[2]; Rabossi A[1]; Radi R[3]; Gevorkian G[4]; Cuello AC[5]; Morelli L[1] [1]FIL-IIBBA CONICET. [2]FMed, UBA. [3]CEINBIO, UdeLaR. [4]UNAM. [5]McGill University E-mail: pmadami@leloir.org.ar

Alzheimer's disease (AD) is the leading cause of dementia in older adults and represents a serious medical, social and economic problem. Although diet is a modifiable risk factor for AD, the mechanisms linking peripheral metabolism and cognition remain unclear. To address this question, we have chosen McGill-R-Thy1-APP transgenic rats (Tg(+/-) that mimic presymptomatic AD pathology. Wild-type and Tg(+/-) rats were exposed from 35 days to 6 months of age to a standard diet or a Western diet (WD), high in saturated fat and sugar. Our results of peripheral and hippocampal biochemical analysis show that WD induced a metabolic syndrome and decreased presynaptic bioenergetic parameters. Furthermore, cognitive tests, ELISA multiplex, Western blot, immunohistochemistry and quantitative RT-PCR results indicate that WD worsened cognition, increased hippocampal levels of oligomeric and monomeric Aβ species (38/40/42), promoted deposits of N-terminal pyroglutamate-Aβ in CA1 pyramidal neurons and interneurons, reduced neuronal resilience and increased nitrated proteins in Tg(+/-) rats. Our results support the concept that diet-induced metabolic dysfunction may contribute as a “second hit” to impair cognition in the presence of early Aβ pathology, reinforcing the relevance of optimizing fat and sugar consumption for the prevention of AD, at least in people with genetic risk factors.

PLANT BIOCHEMISTRY AND MOLECULAR BIOLOGY

PL-C01

MITOCHONDRIAL CONTRIBUTION TO BASAL PLANT DEFENSES VIA PROLINE DEHYDROGENASE (PRODH)

Fabro G, Rizzi YS, Alvarez ME. CIQUIBIC-CONICET, DQB-FCQ, Univ. Nac. Córdoba. E-mail: gfabro@fcq.unc.edu.ar

The contribution of mitochondria to defense programs against bacterial pathogens has been mostly studied under cell death-triggering conditions. Conversely, its influence over defense programs that trigger broad-spectrum resistance in the absence of cell death has not been established yet. To assess this issue, we monitored PTI (PAMP-triggered immunity) features in wild type tissues treated with inhibitors of the mitochondrial electron transport chain (mETC), and mutants lacking the mitochondrial proline catabolic enzyme ProDH. We found that non-lethal levels of antimycin A or rotenone producing mild mETC alterations, strongly impair PTI. In addition, the absence of ProDH1 or ProDH2 isoenzymes reproduces most of these PTI defects. Major differences were observed in the generation of reactive oxygen species (ROS) and deposition of callose at the cell wall. Based on these results we used a bacterial flagellin-derived peptide (flg22) to perform a detailed investigation of the requirement of ProDH for ROS generation by the membrane NADPH oxidase homolog D (RBOHD). Surprisingly, the RBOHD function was sensitively dependent on the ProDH activity.

PL-C02

CHLOROPLAST REDOX STATUS MODULATES GENOMEWIDE STRESS RESPONSES IN SOLANACEOUS PLANTS

Pierella Karlusich JJ[1],Zurbriggen M[1],Shahinnia F[2],Hosseini S[2],Sonnewald S[2], Sonnewald U[2],Hajirezaei MR[2], Carrillo N[1]. [1]Instituto de Biología Molecular y Celular de Rosario (UNR-CONICET) [2]IPKGatersleben, Germany. Email: pierella@ibr-conicet.gov.ar

Chloroplasts are one of the main sources of reactive oxygen species (ROS) in plants. Flavodoxin (Fld) is a photosynthetic protein with antioxidant properties. Although its gene is absent in the plant genome, its expression in transgenic plants

confers increased tolerance to different environmental stresses. These plants are an interesting system to study the role of chloroplast redox status and ROS during plant stresses. Thus, we investigated the transcriptomic responses to Fld expression in two members of the economically important Solanaceae family under different stress assays: 1) Fld - expressing potato plants were subjected to drought, resulting in a less wilted phenotype than their wildtype siblings, 2) Fld expressing tobacco plants were infected with the non-host bacterium *Xanthomonas campestris* pv. *vesicatoria*, showing a ~48 hdelay in the development of localized cell death in inoculated leaves. In both cases, Fld modulated many genes involved in hormone-based pathways, signal transduction, transcriptional regulation and stress responses. Interestingly, ~5% of leaf-expressed genes were affected by Fld expression *per se* in both tobacco and potato under normal conditions. The results provide a genome-wide picture illustrating the relevance of chloroplast redox status on biotic and abiotic stress responses and suggest new targets to generate stress tolerance to solanaceous.

PL-C03

REGULATION OF CENTRAL METABOLISM BY TREHALOSE 6-PHOSPHATE

Figueroa C[12],Feil R[1], Ishihara H[1], Krause U[1], Hoehne M[1], Encke B[1], Stitt M[1], Lunn JE[11]MPI of Molecular Plant Physiology, Golm, Germany. [2]IAL, UNL-CONICET, Santa Fe, Argentina. Email: carfigue@fbcb.unl.edu.ar

Trehalose 6-phosphate (Tre6P) is an important signal metabolite linking plant carbon metabolism with growth and development. The Tre6P-sucrose nexus model postulates that Tre6P regulates sucrose levels within an appropriate range and *vice versa*. To test this model, we investigated short-term metabolic responses to ethanol-induced increases in Tre6P levels in *Arabidopsis thaliana* plants expressing the *Escherichia coli* Tre6P synthase. Increased Tre6P levels led to a transient drop in sucrose content, post-translational activation of both nitrate reductase and phosphoenolpyruvate carboxylase, and increased levels of organic and amino acids. Radio ($^{14}CO_2$) and stable-isotope ($^{13}CO_2$) labelling experiments in plants with elevated Tre6P showed no changes in photoassimilate export rates, but increased labelling of organic acids. These results suggest that high Tre6P levels divert carbon away from sucrose by stimulating carbon fluxes into organic acids to provide C-skeletons for amino acid synthesis and simultaneously stimulate nitrate assimilation. These findings are consistent with the Tre6P-sucrose nexus model and implicate Tre6P in regulating interactions between carbon and nitrogen metabolism in plants.

PL-C04

UNRAVELING THE CONTRIBUTION OF NADP-MALIC ENZYME 1 TO ALUMINUM STRESS RESPONSE IN ARABIDOPSIS ROOTS

Badia MB, Gerrard Wheeler MC, Andreo CS, Drincovich MF.CEFOBI, FCByF, UNRosario, Argentina E-mail: badia@cefobi-conicet.gov.ar

Malate plays a fundamental role in plant aluminum tolerance since its Al-binding capacity. In this work we analyze the participation of *Arabidopsis thaliana* NADP-malic enzyme 1 (NADP-ME1) in root malate metabolism and Al response. Arabidopsis insertional mutant plants lacking *NADP-ME1* showed enhanced tolerance to Al, evidenced by a lower inhibition of root elongation compared to wild type, in presence of toxic Al concentrations. Additionally, qRT-PCR analysis showed a decreased expression of *NADP-ME1* gene in wild type seedlings after 3 hours of Al treatment. The malate levels in roots and exudates after short Al treatments were similar in *nadp-me1* compared to wt, although a significant increase of intracellular malate was observed after a long exposure to Al only in the knockout line. The H_2O_2 content and the transcript levels of several Al tolerance related genes were analyzed in both plant types exposed to Al, as well as the response to glutamate, amino acid implicated in Al stress signal transduction. The results suggested that NADP-ME1 could be involved in regulating the levels of malate in the cytosol of the root apex and its loss may result in an increase in the content of this organic acid. Furthermore, this isoform may be affecting the signal transduction processes which lead to root growth inhibition, such as the generation of ROS or other signaling molecules.

PL-C05

INFLUENCE OF SINAL7 IN VEGETATIVE PARAMETERS IN ARABIDOPSIS

Peralta DA, GomezCasati DF, Busi MV. CEFOBI CONICET Fac Cs Bioquimicas y Farmacéuticas, Universidad Nacional de Rosario E-mail: peralta@cefobi-conicet.gov.ar

Seven in absentia like 7 gene (At5g37890, SINAL7) from *Arabidopsis thaliana* encodes a RING finger protein belonging to the SINA superfamily with E3 ubiquitin ligase activity. In previous work we described the biochemistry characteristics of SINAL7 protein and its self-ubiquitination capability *in vitro*. Moreover, previous evidence led us to propose the participation of SINAL7 in a hypothetical signaling pathway together with cytosolic glyceraldehyde-3-phosphate dehydrogenase in Arabidopsis. In this work we face the study of SINAL7 *in vivo* in Arabidopsis knockout and overexpressing SINAL7 plants to find out its influence on plant physiology by the phenotypic analysis of the plants. Results showed that SINAL7 may be participating in the regulation of several vegetative parameters including biomass content, senescence and drought tolerance. In summary, we found that different levels of SINAL7 in Arabidopsis may alter plant biomass. In addition, we describe the possible compensatory effect of SINAL7 counterparts in knockout mutant sinal7 plants. Finally, we show the influence of SINAL7 in the complex process of senescence and in drought tolerance

PL-C06

SCF E3 LIGASE REDOX REGULATION: IMPACT ON HORMONAL SIGNALINGS

Iglesias MJ, Terrile MC, Casalongue CA Instituto de Investigaciones biológicas IIB-CONICET-UNMDP E-mail: majoi84@hotmail.com

In plants, the ubiquitin-proteasome system action has been associated to the regulation of hormone biosynthesis, transport and perception providing a direct mechanism to modulate intensity and duration of different hormonal signalings. Ubiquitin is covalently attached to substrate proteins through the action of a sequential cascade of three enzymes consisting of E1, E2, and E3. The SCF complex is the best characterized multi-subunit ubiquitin E3 ligase. In SCF complex, SKP1 acts as a bridge between CUL1 and the F-box proteins that mediate substrate recruitment. F-box are able to associate with SKP1 in an interchangeable manner to form diverse SCF complexes with different substrate specificities. Several phytohormone receptors are F-box proteins in SCF complexes regulating auxin, jasmonates and gibberellins signalings. The exchange of F-box substrate adapters from SCF complex must be required during changing cellular conditions. In this work, we present data supporting posttranslational redox regulation of different SFC members and their impact on SCF complex assembly, and hormonal-dependent physiological processes. Supported by ANPCyT, CONICET and UNMdP.

PL-C07

INSIGHT INTO DIVERSIFICATION AND EVOLUTION OF HD-ZIP I TRANSCRIPTION FACTORS IN STREPTOPHYTES

Romani FA, Chan RL, Moreno JE. Instituto de Agrobiotecnología del Litoral (IAL-UNL-CONICET). E-mail: fromani@santafe-conicet.gov.ar

Streptophytes dispersion and evolution onto land was accompanied by an increment in the number of genes encoding transcription factors (TFs). This rise in TFs diversity suggests that they might have played a fundamental role in the adaptation of plants to an environment with less water availability. Homeodomain-leucine zipper I (HD-Zip I) is a TF family whose members are involved in drought responses. In Arabidopsis, this family has 17 members and it was proposed that they have redundant functions. Until now, HD-Zip I were identified only in the land plant lineage. Here, we report the identification of HD-Zip I genes in aquatic charophytes genomes. We also found a single common ancestor in land plants. Duplication events occurring in specific plant divisions might explain the emergence of current clades of HD-Zip I in angiosperms, likely due to neo-functionalization. We also identified a couple of HD-Zip I gene-loss events related to monocot plants that have moved back to aquatic environment. To better understand the diversification and evolution of HD-Zip I TFs, we are now using the livewort *Marchantiapolymorpha*, which occupies a basal position in the evolution of land plants. Marchantia genome also has a low genetic redundancy in regulatory genes and encodes a unique HD-Zip I that could be key to understand the role of these genes during the transition to land.

PL-C08

AN OPEN READING FRAME PRESENT IN THE 5'UTR OF THE ARABIDOPSIS ATHB1 GENE REPRESSED ITS TRANSLATION

Ribone PA,Capella M, Chan RL. Instituto de Agrobiotecnología del Litoral (UNLCONICET). Santa Fe. E-mail: pamela.ribone@santafe-conicet.gov.ar

AtHB1 is an Arabidopsis transcription factor (TF) belonging to the HD-Zip I subfamily. It was previously demonstrated that this TF interacts with AtTBP2 to exert its function, acting downstream of PIF1. The AtHB1 gene is expressed in hypocotyls and roots to regulate genes involved in the elongation and synthesis of the cell wall. Here we show that bioinformatic analyses considering AtHB1 homologues in several plant species resulted in the detection of a conserved upstream Open Reading Frame (uORF) in the 5'UTR region of these genes, named CPuORF33. These uORFs exhibit a higher degree of conservation in the aminoacidic sequences than in the nucleotide ones, and they do not overlap with the mORF (main ORF). Generation of different constructs, bearing the native or/and the mutated uORF, used to transform Arabidopsis plants allowed us to demonstrate that the CPuORF33 represses the mORF translation but not transcription, independently on the mORF nature. This regulation occurs in cis, probably through a ribosome stalling mechanism. On the other hand, AtHB1 overexpression is also repressed by small RNAs in Arabidopsis. Altogether, our results reveal a novel mechanism by which the expression of AtHB1 (and that of its homologues in other plant species) is tightly regulated in order to avoid aberrant phenotypes observed when the TF is overexpressed leading to sterile plants.

PL-C09

CYTOCHROME C MODULATES PLANT GROWTHRATE AND THE ACTIVITY OF THE GIBBERELLINPATHWAY

Racca S,Welchen E,Gonzalez DH. Instituto de Agrobiotecnologia del Litoral (IAL-UNL-CONICET).Santa Fe, Argentina.E-mail:sofia.racca@hotmail.com

We studied the effect of reducing the levels of the mitochondrial electron carrier cytochrome c (CYTc) in plants. Double knockdown mutants in both genes encoding CYTc in *Arabidopsis thaliana* show a severe delay in vegetative growth and developmental rate. Additionally, CYTc deficiency causes starch and glucose accumulation indicating that these plants accumulate reserves instead of using them for growth. Treatment of mutants with the gibberellin GA47abolishes the developmental delay, suggesting that it is associated to GA deficiency. Transcriptional analyses show that the expression of several genes involved in GA homeostasis is altered in mutant plants, while the levels of DELLA proteins, repressors of GA signaling, are increased. In addition, plants with increased CYTc levels show accelerated growth and reduced

levels of DELLA proteins. We propose that hormone regulation of growth is coupled to the activity of components involved in mitochondrial energy metabolism.

PL-C10

TCP15 CONNECTS GIBBERELLIN AND AUXINPATHWAYS DURING STAMEN FILAMENTELONGATION IN ARABIDOPSIS

Gastaldi V, Lucero LE,Gonzalez DH. Instituto de Agrobiotecnologia del Litoral (IAL-CONICET-UNL)Santa Fe, Argentina. Email: vgastaldi@santafe-conicet.gov.ar

The TCP transcription factor family is specific of plants.*Arabidopsis thaliana* has 24 TCP proteins divided into classes Iand II. In this work, we characterized the role of TCP15, amember of class I, in stamen development. Plants that express afusion of TCP15 to the EAR repressor motif develop shorterstamen filaments, while plants that express TCP15 under thecontrol of the 35SCaMV promoter show longer filamentscompared to wild-typeplants. Transcript levels of SAUR63, anauxin response gene implicated in filament elongation, arereduced in TCP15-EARand increased in 35S:TCP15 plants.Filament elongation is also promoted by gibberellins (GA) andmutants in GA biosynthesis genes have short stamen filaments.Inhibition of GA synthesis decreases the expression of SAUR63and overexpression of TCP15 rescues the short stamenphenotype of GA deficient plants. In addition, plants thatoverexpress the transcription factor KNAT1 have shorterfilaments while mutants in the corresponding gene show longerfilaments. KNAT1 is a known repressor of GA synthesis andoverexpression of TCP15 rescues the short stamen phenotypeof 35S:KNAT1 plants. We conclude that TCP15 modulates thecrosstalk between GA levels and auxin responses during stamen development and that KNAT1 is an upstream regulatorof the process.

PL-C11

POST-TRANSLATIONAL REGULATION OF MICRO RNA BIOGENESIS

Achkar NP, Manavella PA Instituto de Agrobiotecnología del Litoral (IAL), UNLCONICET Email: natalia.achkar@gmail.com

Gene regulation is an important mechanism used by plants to face unfavourable environmental conditions, and micro RNAs (miRNAs) are central players in such process. The miRNAs, small 21nucleotide RNAs molecules, post-transcriptionally control gene expression. In order to achieve a balance between gene expression and silencing, both the miRNA biogenesis and action are tightly regulated. In this sense, posttranslational modifications of miRNA biogenesis cofactors are essential for the proper functionality of the pathway. HYPONASTIC LEAVES 1 (HYL1), a miRNA biogenesis cofactor, is post-translationally regulated by two mechanisms. On one hand, HYL1 activity depends on the phosphorylation of its RNA and protein-protein interaction domains. On the other, both its stability and degradation are defined by an undefined mechanism controlled by the environmental light conditions. Here, we report that HYL1 phosphorylation pattern changes depending on the growth conditions. Small RNA blots allowed us to find out that the environmental controlled changes in the HYL1 phosphorylation influence its activity impacting the mature miRNAs production. In a similar way, western blot and confocal microscopy allowed us to identify some particular conditions where the stability of HYL1 is impaired.

PL-C12

INTEGRATION OF LIGHT AND TEMPERATURE CUES IN PLANT DEVELOPMENT

Legris M[1], Costigliolo Rojas MC[1], Vierstra R[2], Casal J[1] [31]Fundación Instituto Leloir, IBBA–CONICET. [2]Washington University.[3]IFEVA, FAUBACONICET. E-mail: mlegris@leloir.org.ar

Plants, as sessile organisms, depend on their capacity to perceive and respond to changes in environment in order to survive and reproduce. Among ambient factors, light and temperature are undoubtedly the most relevant for plant development. For example, increasing light signaling inhibits hypocoyl elongation whereas increasing temperature (below the optimum) promotes hypocoyl elongation. The aim of this work is to analyze the combined effects of these signals on plant morphogenesis. Seedlings growing in nature can be shaded by taller plants, with a consequent reduction in radiation which lowers air and leaf temperature. Also, since chlorophylls absorb mainly in the blue and red portions of the spectrum, light under a canopy is enriched in green and far red wavelengths compared to sunlight. Changes in the red/far red ratio and red light intensity are perceived mainly by phytochrome B (phyB), one of the five members of the phytochrome family in Arabidopsis. To assess the effect of light and temperature changes caused by neighbors on plant morphogenesis transgenic lines of Arabidopsis carrying phyB variants of different stability were grown under a wide range of light conditions and temperatures. Hypocotyl elongation and the subnuclear distribution of phyBGFP were recorded. This data allowed us to develop a model which predicts plant growth in different light and temperature conditions.

PL-C13

PAP-SAL1 RETROGRADE PATHWAY IS INVOLVED IN IRON HOMEOSTASIS IN *ARABIDOPSIS THALIANA*

Balparda M[1], Estavillo G[2], Gomez-Casati DF[1], Pagani MA[11]CEFOBI (UNR-CONICET), Rosario, Argentina. [2]CSIRO Plant Industry, Canberra, Australia. E-mail: balparda@cefobi-conicet. gov.ar

Nuclear gene expression is regulated by a diversity of retrograde signals that travel from the organelles to the nucleus in a lineal or classical model. One such signal molecule is 3´-phosphoadenisine-5´-phosphate (PAP), the levels of PAP *in vivo* are regulated by a phosphatase enzyme SAL1/FRY1 located in chloroplast and mitochondria. This metabolite inhibits the

action of a group of enzymes called exoribonucleases (XRNs), which participate in the regulation of the posttranscriptional gene expression. Transcriptome analysis of *Arabidopsis* mutant plants in PAP-SAL1 pathway revealed that the ferritin genes *AtFer1, AtFer3, and AtFer4* are up regulated in these genetic backgrounds, thus establishing a link between the PAP retrograde signaling pathway and the regulation of Fe homeostasis genes. In this work, we studied Fe homeostasis in *sal1* and *xrn* mutants, showing differences in the expression of genes implicated in Fe uptake and storage, we also observe divergences in the enzyme activities concerned in Fe uptake, comparing with wild type. In Fe deficiency conditions, *sal1* and *xrn* mutants grew well while wild type plants were clearly affected. These mutants presented a miscommunication between the root and the shoot, up-regulating Fe acquisition genes even when the nutritional demands are fulfilled.

PL-C14

IMPORTANCE OF THE PRECURSOR PRIMARY AND SECONDARY STRUCTURE DURING MICRORNA PROCESSING IN PLANTS

Rojas A, Bresso E, Schapire A, Moro B, Mateos J, Palatnik J. Instituto de Biología Molecular y Celular de Rosario (IBR) E-mail: arojas@ibr-conicet.gov.ar

MicroRNAs are 2022 nt small RNAs that play a role as posttranscriptional gene regulators. They recognize target sequences in longer RNAs by base complementarity and guide them to cleavage or translational arrest. MicroRNAs are generated from longer precursors that harbor a fold-back structure with the microRNA located in one of arms. In plants, microRNA precursors are completely processed in the nuclei by DICER-LIKE1 (DCL1). Artificial microRNAs can be generated by modifying the microRNA sequence in an endogenous precursor. Currently, artificial microRNAs are widely use in a broad range of species to inactivate specific target genes. Understanding microRNA precursor processing is then also important to improve artificial microRNA technologies. Here, we performed a random mutagenesis approach in Arabidopsis to identify mutations that improve the processing of the precursor of miR172, a microRNA that regulates flowering time. We found that a single base change increased by several folds the amount of microRNA generated by the precursor. Furthermore, a systematic analysis revealed the importance of the secondary and also the primary sequence at specific positions of the miR172 precursor. The results provide new insights into the molecular basis of microRNA processing in plants and provide elements to design more efficient artificial microRNAs.

PL-C15

BACTERICIDAL AND CYTOTOXIC ACTIVITIES OFPOLYPHENOL EXTRACTS FROM ANDEAN ANDINDUSTRIAL POTATOES

Lanteri ML[1], Silveyra MX[1],Damiano R[12], Andreu AB[1] [1]Instituto de Investigaciones Biológicas, UNMdP, CONICET .[2]INE "Dr. Juan H. Jara" Mar del Plata. Email:lanteri@gmail.com

Potatoes (*Solanum tuberosum*) are a good source of dietary antioxidant polyphenols. This study investigated the *in vitro* antioxidant, bactericidal and cytotoxic activities of the polyphenols present in tubers of three Andean and one industrial potato varieties. Both phenolic acids content and antioxidant activity were higher in skin extracts than in flesh ones, being chlorogenic acid (CGA) the most abundant phenolic acid. Extracts from Andean Moradita flesh and from industrial Summer side skin showed bactericidal activity against E. coli ATCC 25922. Both extracts have high absolute content of CGA, presence of ferulic acid and absence of pigmentation. In contrast, no bactericidal effects were found against pathogenic *E. coli* O157. Positive control with gentamicin and commercial CGA resulted in inhibition of bacterial growth. We also showed that all extracts exerted a dose dependent cytotoxic effect in SH-SY5Yhuman neuroblastoma cells. Skin extracts were more potent than flesh ones, and commercial CGA treatments compromised SH-SY5Ycell viability. On the whole, results demonstrate that extracts with similar phenolic acid level and/or composition do not exert similar antioxidant and/or biological activity. These findings suggest that the activity of potato extracts is a combination of various bioactive compounds and contribute to the revalorization of potato as a functional food.

PL-C16

REGULATION OF THE PLANT MICRO RNA MACHINERY BY A MSS47-MEDIATED EPIGENETIC MECHANISM

Ré DA, Manavella PA. Instituto de Agrobiotecnología del Litoral – UNL-CONICET SantaFe, 3000, Santa Fe, Argentina. E-mail: delfina.a.re@gmail.com

Micro RNAs (miRNA) are 21 nucleotides molecules essential for post-transcriptional gene silencing. These molecules are generated from a structured RNA precursor and then incorporated into the RNA induced silencing complex (RISC).DICER-LIKE1, HYPONASTIC LEAVES 1 and SERRATE are the main proteins involved in miRNA biogenesis but several new cofactors have being described recently. After processing of the miRNA precursors, ARGONAUTE 1 (AGO1) binds the mature miRNAs and inhibits targeted-mRNA translation or induces its cleavage. Using a forward genetic approach we have isolated novel mutants deficient in miRNA activity, from which several cofactors of the pathway, such as CPL1, RCF3 and the THO/TREX complex, have been recently described. Here we describe MIRNA-SILENCINGSUPPRESSED 47 (mss47), another miRNA deficient mutant identified in our genetic screening. Small RNA blots and RT-qPCR showed that mss47 mutants present altered level of miRNAs and targets' mRNAs. MSS47 codifies a protein with a histone-methyl transferase domain. This protein does not directly regulate the transcripts levels of MIRNA genes or its targets. Our experiments show that MSS47epigenetically regulates the abundance of essential

factors of the miRNA pathway. Such depletion in the miRNA machinery impairs the production and activity of miRNAs and, therefore, the gene silencing and plant development.

PL-C17

PHYTOCHROME B REGULATES SYSTEMICSIGNALING OF DEFENSE RESPONSE INARABIDOPSIS

Moreno JE, Etchevers L Laboratorio de Biotecnología Vegetal, Instituto de Agrobiotecnología del Litoral (UNL-CONICET) E-mail: javier.moreno@santafe-conicet.gov.ar

Phytochromes are a conserved group of photoreceptors that control multiple aspects of plant architecture and health. Phytochromes (Phy) oscillate between an active and inactive state depending on the red (R, 680 nm) to far red (FR, 730 nm)ratio of the incoming light. The R:FR ratio is translated into information by Phy to let the plant perceive and out compete future competitors through the initiation of the shade avoidance syndrome (SAS). PhyB is the main phytochrome controlling SAS. Interestingly, PhyB inactivation or mutation in Arabidopsisis accompanied by suppression of immune responses leading to enhanced susceptibility to insect damage and fungal infection. Given that PhyB controls the systemic differentiation of stomata and systemic development of the photosynthesis apparatus, we wondered if PhyB might also regulate the systemic induction of the defense response controlled by the phytohormone jasmonate (JA). To address this question we used a set of transgenic lines with tissue-specific expression of PhyB or inducible expression of PhyB. In order to establish the PhyB contribution to the systemic defense response, we performed bioassays based on the infection of the necrotrophic fungi *Botrytis cinerea* and monitored total phenolic contents and the expression of marker genes in leaves. The results will be discussed in the context of the current knowledge of the field**.**

PL-C18

PLANT NATRIURETIC PEPTIDES IMPROVE PLANTRESISTANCE DURING BIOTIC STRESS

Grandellis C, Ficarra F, Garavaglia BS, Gottig N, Ottado J Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR) E-mail: grandellis@ibr-conicet.gov.ar

Plant natriuretic peptides (PNPs) are extracellular, systemically mobile molecules that are involved in the modulation of salt and water homeostasis and AtPNPA from *Arabidopsis thaliana* has been extensively studied. Distinctively, the bacterial pathogen of citrus plants, *Xanthomonas citri* subsp. *Citri* (Xcc) contains a *PNP*-like gene (*XacPNP*). Both peptides, AtPNPA and XacPNP induce similar physiological responses when applied on plant tissue, including stomatal opening and photosynthetic efficiency improvement. *A. thaliana-Pseudomonassyringae* pv. *Tomato* (Pst) patho system and its genetic resources allowed us to analyze the role of XacPNP and AtPNPA in *A. thaliana* during infection. To this aim, *A. thaliana* transgenic lines were generated overexpressing *XacPNP* and *AtPNPA*, and RNA interference lines silencing endogenous *AtPNPA* were also obtained. Overexpressing *PNPs* lines showed enhanced resistance to Pst, while *PNP*-deficient plants were more susceptible. Moreover, pretreatment of *A. thaliana* leaves with XacPNP before Pst infection resulted in increased resistance evidenced by higher remnant chlorophyll, lower pathogen survival and induction of defense associated genes. Our results state a role for PNPs during plant biotic stress improving plant performance under stressful conditions.

PL-C19

A GLYCINE RICH PROTEIN IS INVOLVED IN*XANTHOMONAS CITRI* SUBSP. *CITRI*-PLANTINTERACTION

Vranych C, Piazza A, Ottado J, Gottig N. Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET-UNR),Ocampo y Esmeralda, Rosario E-mail: vranych@ibr-conicet.gov.ar

The Type III Secretion System (TTSS) is critical for pathogenicity and Hypersensitive Response (HR) induction by phytopathogens as *Xanthomonas citri*s ubsp. *citri* (Xcc): causal agent of citrus canker. HrpE protein is a principal component of TTSS and previously we characterized that this protein induces defense responses and HR in several plants. Moreover, by yeast two hybrid assays we had identified a citrus Glycine Rich Protein (GRP) as a putative protein involved in HrpE recognition. Interestingly, GRPs have a key role in plant defense mechanisms in several plant species. In this work, Bimolecular Fluorescence Complementation assays confirmed HrpE-GRP interaction *in vivo*. Also, we studied the response of HrpE intransgenic *Arabidopsis thaliana* knock outs in a GRP endogenous gene that is the closest homolog to the citrus GRP. These mutant GRP lines showed absence or slight HR in leaves infiltrated with HrpE recombinant protein compared to wild type plants. Furthermore, cell death, callose deposition and the expression of defense genes as PR1 were decreased in these transgenic lines treated with HrpE. These results suggest that GRP is involved in HrpE recognition and signal transduction events triggering plant defenses against Xcc. Our results indicate for the first time a role of plant GRP in citrus canker disease. This study enhances the understanding of Xcc infection process.

PL-C20

DESIGN OF A GFP-BASED NON-INVASIVE BIOSENSOR TO DETERMINE $NADP^+$(H) REDOX STATE IN LIVING CELLS

Molinari PE[1], Zurbriggen M[2], Bustos-Sanmamed P[3], Krapp AR[3], Carrillo N[31]FBIOyF UNR, [2]Inst. Synthetic Biol. Heinrich Heine Univ. Dusseldorf Alemania, [3]IBR-CONICET Argentina E-mail: pmolinari@fbioyf.unr.edu.ar

One key component of central metabolism in all life forms is $NADP^+$(H). Current methods for $NADP^+$(H) determination in biological samples are hampered by lack of sensitivity and reproducibility, precluding studies of redox changes in living cells. The recent development of redox sensitive green fluorescent proteins (roGFPs) as genetically encoded

probes allowed spatiotemporal detection of thiol-containing redox metabolites in vivo. We designed a biosensor to determine the cellular redox state of $NADP^+(H)$, by fusing roGFP to the coding region of rice $NADP^+(H)$ thioredoxin reductase C (NTRC) via spacer arms of variable lengths (30 and 45 residues). NTRC allows transduction of redox signals from the world of pyridine nucleotides to that of thiol-disulfides. The NTRC-roGFP fusions display reversible ratiometric fluorescence changes in response to the redox potential of $NADP^+(H)$. *In vitro* characterization of both fusion proteins indicated that ratiometric values varied with the redox potential of $NADP^+(H)$ as predicted by the Nernst equation, permitting its accurate determination by measuring the oxidation state of the probe. As a proof-of-concept test we transformed *Escherichia coli* cells and observed fluorescence changes by confocal microscopy in response to the redox state of the organism. This biosensor can be used to study the $NADP^+(H)$ redox poise of cells and organelles in real time.

PL-C21

HEAT STRESS INDUCES FERROPTOSIS LIKE CELL DEATH IN PLANTS

Distéfano A[1], Martin M*[1], Córdoba J[1], Bellido A[1], Roldán J[1], Bartoli C[2], Zabaleta E[1], Fiol D[1], Stockwell B[3], DixonS[3],PagnussatG[11]IIB-CONICET-UNMdPMar del Plata, Argentina [2]InFIVE-CONICET-UNLPLa Plata Argentina[3]DeptBiological Sciences, Columbia University, NY USA *equal contribution of both authors E-mail:adistefa@mdp.edu.ar*

In plants, regulated cell death plays critical roles during development and is essential for plant-specific responses to abiotic and biotic stresses. However, the molecular mechanisms underlying plant cell death remain unclear. In this work, we examined whether ferroptosis, an iron-dependent, oxidative process recently described to occur in animal cells, could be relevant to cell death in plants. Although ferroptotic cell death was not involved in reproductive or vascular development, it was implicated in heat-shock-induced regulated cell death. Analyses of heat shock treated (HS) Arabidopsis roots using DIC and TEM microscopy showed a specific morphology associated to cell death. Biochemical features that are specific for animal ferroptosis are induced in HS-Arabidopsis roots, such as the iron dependent accumulation of ROS and lipid ROS, and the depletion of glutathione and ascorbic acid. The study of the expression pattern of several genes related to cell death processes in plants and animals showed that a recently described gene (named KOD), which encodes a short peptide that regulates plant cell death is specifically regulated in HS-Arabidopsis roots. Although additional factors involved in the ferroptosis pathway remain to be identified in plants, many characteristics are conserved between plants and animal cells suggesting that ferroptosis is a conserved form of cell death.

STRUCTURAL BIOLOGY

SB-C01

GENERATION OF NANOBODIES AS A TOOL FOR STRUCTURAL BIOLOGY

Alzogaray VA, Goldbaum FA. Fundación Instituto Leloir. E-mail: valzogaray@leloir.org.ar

A promising approach to increase crystal formation and to improve diffraction quality is the use of crystallization chaperones. Nanobodies are exquisite chaperones for crystallizing complex biological systems such as membrane proteins. Nanobodies are the smallest and most stable single-domain fragments with the full antigen-binding capacity and that naturally occur in camelids. Here we describe a general methodology for the generation of nanobodies to be used as crystallization chaperones for the structural investigation of soluble and membrane proteins. We have set up a general pipeline to obtain nanobodies against different immunogens, particularly those of clinical relevance. Some of them are membrane proteins resuspended in detergent buffers and lipid vesicles for preserving their native conformation. The strategy includes the use of phage display technology to select the nanobodies, a step that was carried out with different methods to coat the antigens. We employed solid surface of an ELISA plate and nickel coated plate that allows to spatially orient the target protein. As a result, after two rounds of selection, phages that recognize conformational epitopes were obtained. The nanobodies are expressed in the periplasmic space and sixty specific clones were selected for sequencing by binding assays and their ability to avoid aggregation.

SB-C02

STRUCTURAL AND FUNCTIONAL STUDIES OF THE NTRX RESPONSE REGULATOR, A DIMERIC ATP BINDING PROTEIN

Fernández I[1], Cornaciu I[2], Carrica MC[1], Uchikawa E[2], Márquez JA[2], Goldbaum FA[1]. [1]Fundación Instituto Leloir, IIBBA (CONICET). [2]EMBL Outstation Grenoble E-mail: ifernandez@leloir.org.ar

Bacteria need to adapt to environmental changes in order to survive. Among the mechanisms employed for this task are the two-component systems (TCS). They are formed by a histidine kinase (HK) that autophosphorylates upon perception of a stimulus and transfers the phosphoryl group to the second component, the response regulator (RR), which modulates gene transcription. Our group has been interested in a TCS formed by the HK NtrY and the RR NtrX, which has been implicated in the detection of low oxygen tension in *Brucella abortus*. NtrX has a REC, a central AAA+, and a DNA binding domain. Previous studies allowed us to obtain the crystal structure of the full-length protein and to analyze the DNA binding. In this opportunity, we have examined some characteristics related to the AAA+ domain in order to gain insights into how NtrX works. We found that this RR is able to bind ATP but it cannot hydrolyze the nucleotide.

Furthermore, we solved the structures obtained by soaking NtrX crystals with ATP and ADP, and describe the binding pocket. Also, we found that NtrX is a dimer in solution and that it does not undergo further oligomerization as a consequence of phosphorylation or nucleotide binding. Finally, we have developed a preliminary design for an in-vivo assay with *Caulobacter crescentus* to perform structure-function studies to identify important residues in NtrX mechanism of action.

SB-C03

UNRAVELLING THE LONG-RANGE SIGNALING MECHANISM OF BACTERIOPHYTOCHROMES

Otero LH[1], Klinke S[1], Rinaldi JJ[1], Velázquez F[2], Mroginski M[2], Hildebrandt P[2], Goldbaum FA[1], Bonomi HR[1]. [1]Fundación Instituto Leloir, Argentina. [2]Technische Universität Berlin, Germany. E-mail: lotero@leloir.org.ar

Light-induced reactions allow organisms to adapt to different environmental factors. Bacteriophytochromes (BphPs) are light-sensing proteins found among photosynthetic and non-photosynthetic bacteria that are reversibly photoconverted between a red-absorbing (Pr) and a far-red-absorbing (Pfr) state. Most BphPs share a common architecture consisting of an N-terminal photosensor core module (PCM), which detects the light signal, and a C-terminal variable output module (OM), responsible for transducing this information into a biological effect. To date, it is still not fully understood how structural changes are propagated from the PCM to the OM during the photoconversion Pr-Pfr in the signal transduction event. Here we present the crystal structures of the full-lengh BphP (PCM + OM) from the plant pathogen Xanthomonas campestris (XccBphP) and of its isolated PCM. In the crystals, the full-lengh protein showed a Pr state while the PCM was found to be in the Pfr state. The quaternary assembly reveals a head-to-head dimer in which the OM contributes to the helical dimer interface. In solution, the full-length version behaves as a dimer while the PCM construct is a monomer. Our structural analysis suggests that the long-range signaling in BphPs may involve a kink and a rotation of the OM position via a helical spine movement during the photoconversion Pr-Pfr.

SIGNAL TRANSDUCTION

ST-C01

STRESS GRANULES CONTROL PROTEIN SYNTHESIS AND HAVE A NOVEL LINK TO NEURODEGENERATION

Perez-Pepe M, *KatzMJ, Wappner P, Boccaccio GL. Fundación Instituto Leloir - IIBBA CONICET-E-mail: maperez@leloir.org.ar*

Stress granules (SGs) are cytoplasmic supramolecular aggregates that form transiently in eukaryote cells upon acute stress. SGs belong to a growing family of "liquid organelles", which are membraneless and depend on protein aggregation domains for their formation. SGs contain repressed mRNAs, translation initiation factors and several RNA-binding proteins and are related to a number of abnormal protein aggregates present in neurodegeneration. Their significance to cell survival remains elusive. Here we show that SGs are involved in the control of the translational reprogramming upon stress. Using a puromycin-based method to measure translation in single cells we found that SGs form after the shutdown of protein synthesis and inactivation of eIF2alpha. However, the recovery of translation correlates with SG dissolution, thus suggesting that SG disassembly and release of mRNAs might be an important event to reinitiate protein synthesis. In a RNAi–based screen performed in Drosophila cells we identified 21 positive and 16 negative modulators of SG formation involved in mRNA metabolism and translation and linked to neurodegeneration. We confirmed the role of the vertebrate homologs in mammalian cell lines and we are currently investigating their role in the neuronal stress response in the Drosophila brain.We thank the DRSC, HMS, and ANPCyT, CONICET and UBA, Argentina for funding.

ST-C02,

ROLE OF THE SCAFFOLD PROTEIN STE5 IN THE INTEGRATION OF CDK AND MAPK SIGNALS: A DYNAMIC VIEW

Repetto MV[1], Bush A[1], Winters MJ[2], Pryciak PM[2], Colman-LernerAA[1]. [1]IFIBYNE-CONICET and Departamento de Fisiología, Biología Molecular y Celular, FCEN, UBA, Argentina. [2] MGM UMASS Med. School, USA.E-mail: vrepettor@fbmc.fcen.uba.ar

In *S. cerevisiae*, pheromone activates a GPRC coupled to a MAPK cascade pathway that, among other effects, arrests the cell cycle in G1. However, in cells committed to a new round of mitosis, CDK activity blocks pheromone response. Plasma membrane (PM) recruitment of the mating MAPK scaffold, Ste5, is a key step in pheromone signaling. It is this membrane interaction that is inhibited by CDK activity by phosphorylating residues flanking the Ste5 PM binding domain. Using a quantitative microscopy method to measure protein re-localization over time we studied the early dynamics of Ste5 recruitment in single live cells. We found that Ste5 inhibition by CDK requires negative feedback by the mating MAPK. Further analysis suggested that CDK-mediated inhibition and the Fus3-dependent negative feedback operate via the same set of phosphorylation sites in Ste5. Despite the inhibitory potential of both kinases, proper signal inhibition after cell cycle "start" can only be achieved by collaborative activity of CDK and MAPK. MAPK alone cannot displace Ste5 from the membrane but its activity is required to block MAPK signaling at low levels of CDK activity. We argue that this collaborative effect may be especially helpful in late-G1, when the key cyclins are not yet at peak levels, and that the negative feedback of the MAPK acts as a mechanism to impede improper activation of the mating program.

ST-C03

PROTEIN KINASE A LOCALIZATION IS CRITICAL FOR SPERM CAPACITATION

Stival VC[1],Ritagliati C[1], Luque GM, Baro Graf C[1], Visconti PE, Buffone MG, Krapf D[1]. [1]Laboratory of Cell Signal Transduction Networks, IBR (CONICET-UNR), Rosario, Argentina E-mail: stival@ibr-conicet.gov.ar

Capacitation is the process that renders sperm able to fertilize. It is characterized by a shift in the motility pattern (hyperactivation) and acquisition of acrosome responsiveness. Protein Kinase A (PKA) is known as a key player during capacitation events, coordinating downstream events such as membrane hyperpolarization and hyperactivation. PKA is activated early during capacitation due to a rapid increase of intracellular cAMP. However, current evidence supports the hypothesis that proper localization of the enzyme is also crucial for its regulation, either positioning the kinase in close contact with its substrates or alternatively, refraining contact from them. Here, we address the role of PKA anchoring to AKPAs through the usage of a permeable peptide named st-HT31, to study this type of PKA regulation in capacitation and during acrosome reaction (AR) using mouse sperm. Delocalization of PKA blocked both its activity as well as tyrosine phosphorylation. Moreover, this blockade prevented membrane hyperpolarization of sperm. In this regard, st-HT31 also prevented the acrosome reaction and in vitro fertilization. Worth noticing, even though the biological activity of PKA was affected, the chemical activity of PKA was not impaired, as addressed by in vitro activity of PKA in the presence of st-HT31. Our results uncover a new type of PKA regulation during sperm capacitation.

ST-C04

ESSENTIAL ROLE OF CFTR IN HUMAN SPERM REGULATION OF MEMBRANE POTENTIAL AND PHI DURING CAPACITATION

Puga Molina LP[1], PintoNP[1],Krapf DK[2],Buffone MB[1]. [1]Instituto de Biología y Medicina Experimental [2]Instituto de Biología Molecular y Celular de Rosario E-mail: krapf@ibr-conicet.gov.ar

At ejaculation, mature sperm are not able to fertilize an oocyte. They require to spend a limited period of time in the female reproductive tract and undergo several maturational changes, all grouped under the name of capacitation. From a molecular point of view, one of the first events that occur during capacitation is a HCO3--dependent activation of the atypical soluble adenylyl cyclase ADCY10 which leads to cAMP synthesis and the subsequent activation of PKA. Also, HCO3- triggers alkalinization of the cytoplasm and membrane hyperpolarization. However, the mechanisms by which HCO3- is transported into the human sperm and modulates intracellular pH and membrane potential is not well established. There is evidence that CFTR activity is involved in the human sperm capacitation but how this channel is integrated in the complex signaling cascades associated with this process remains largely unknown. In the present work we have analyzed the extent to which CFTR regulates of intracellular pH and membrane potential during capacitation. We observed that inhibition of CFTR affects HCO3- entrance resulting in lower PKA activity and that cAMP/PKA-downstream events are essential for the regulation of intracellular pH and membrane potential.

ST-C05

ACYL-COA SYNTHETASE 4 (ACSL4) IS PART OF THE ACQUISITION OF ANTICANCER DRUG RESISTANT IN CANCER

Orlando UD, Castillo AF, Solano AR, Maloberti PM, Podestá EJ. Instituto de Investigaciones Biomédicas, INBIOMED (UBA-CONICET), Facultad de Medicina, UBA.E-mail: ulises_orlando@yahoo.com.ar

The most common reason for acquisition of resistance to anticancer drugs is expression of multidrug resistant proteins (MRP) that detect and eject anticancer drugs from cells. For triple-negative breast cancer treatment, there is renewed interest in investigating the role of platinum-derived chemotherapy, however still there is a significant toxic effect of these drugs. Here we demonstrate that sensitivity of different breast cancer cell lines to chemotherapy agents correlates inversely with ACSL4 expression. Using the MCF-7 Tet-Off/ACSL4 model, we show a significant inhibition of cell proliferation at 20 μM of both carboplatin and cisplatin in ACSL4 expressing cells vs. an inhibition at 5 μM and 2.5 μM of carboplatin and cisplatin respectively in control cells. By ACSL4 expression up or down regulation, we demonstrate a regulation of several MRP genes. Combination of the inhibition of ACSL4 activity with cisplatin or carboplatin produces a significant synergistic effect to reduce tumor growth in the triple negative human breast cancer cell line. Strikingly the doses used of both agents did not produce any effect per se, suggesting the probability of reducing the toxic side effects of these agents when used in effective doses. Such a synergistic effect may not only be due to the inactivation of MRP genes but also to the inhibition of parallel pathways supporting tumor survival.

ST-C06

TWO-COMPONENT SYSTEMS IN BACTERIA: HOW IS THE SIGNAL UNIDIRECTIONALLY TRANSMITTED?

Imelio J, Trajtenberg F, Mechaly A, Larrieux N, Buschiazzo A. Molecular & Structural Microbiology Lab, Institut Pasteur Montevideo.

Two-component systems (TCS), the main signaling pathways in prokaryotes, are canonically constituted of a sensor histidine-kinase (HK) and a cognate response regulator (RR). Upon a given environmental stimulus, the HK can autophosphorylate in a conserved His residue using ATP, and then transfer the phosphoryl to a conserved Asp of the RR, who often acts as a transcription factor executing a physiological adaptive response. In the absence of signal, the HK keeps its RR in a dephosphorylated state, acting also as a cognate phosphatase. These pathways have been shown to be highly specific and efficient, with a "His-to-Asp" irreversible phosphoryl pathway. However, more complex TCS termed phosphorelays, rely on shuttle domains that allow phosphate flow to occur bidirectionally (also "Asp-to-His"). By X-ray

crystallography and biochemical *in vitro* assays, this work aims to elucidate how these canonical TCS elicit adaptive outputs through a tightly controlled and unidirectional phosphate flow. We have solved the crystal structures of the study model DesK-DesR complex, of *Bacillus subtilis*, in the phosphatase and phosphotransfer states at atomic resolution, using cytoplasmic DesK mutants known to stabilize different functional states. Structural data here presented contribute to understand how signaling occurs unidirectionally, ensuring the connection between stimulus and adaptive response.