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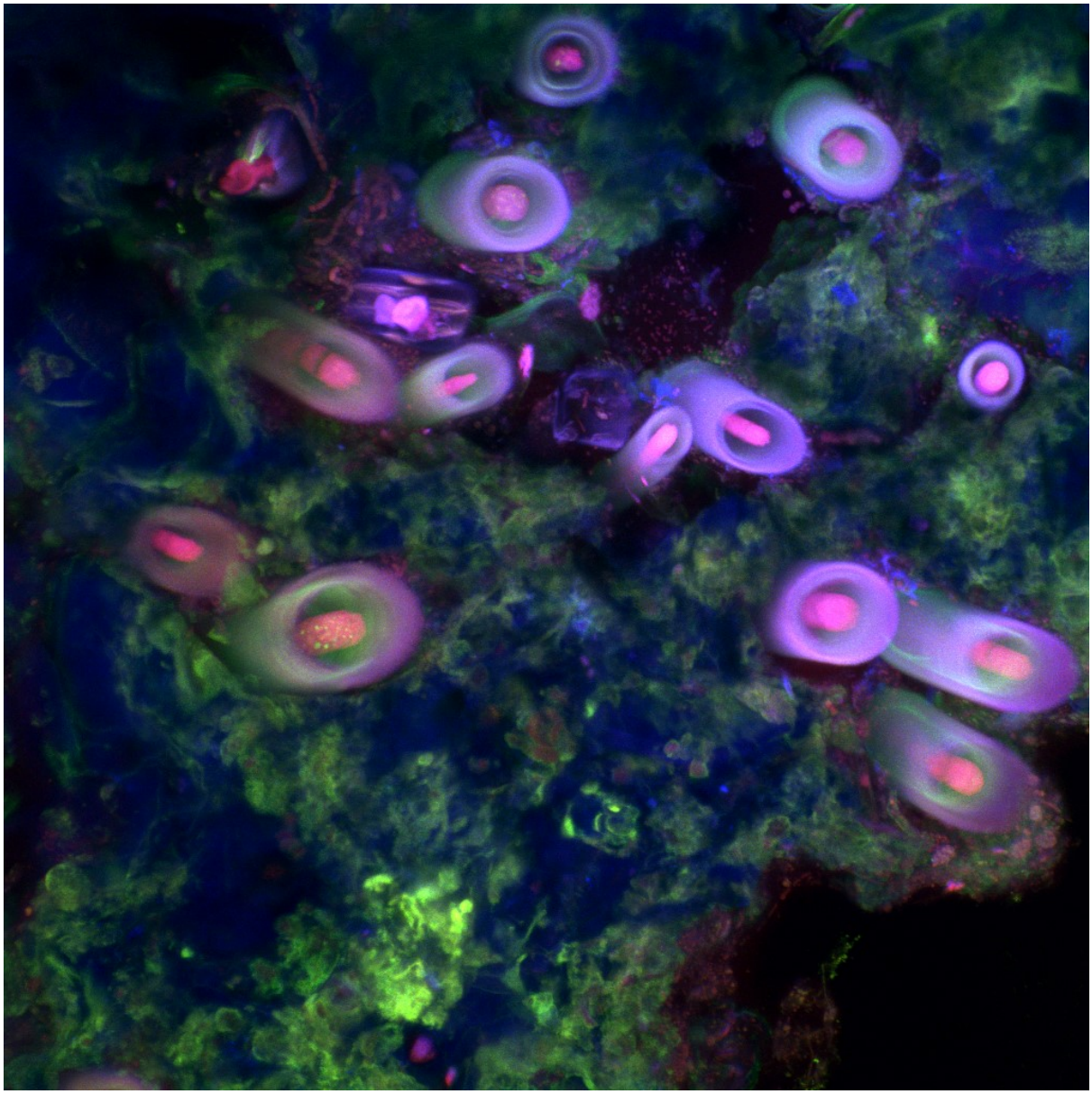
LVI SAIB Meeting – XV SAMIGE Meeting



SAIB-SAMIGE Joint Meeting 2020 – *Online*

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LVI Annual Meeting
Argentine Society for Biochemistry and Molecular Biology
(SAIB)

XV Annual Meeting
Argentinean Society for General Microbiology
(SAMIGE)

SAIB-SAMIGE – Online
Joint Meeting 2020

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DMEM and collected at different times. mRNA levels were quantified by qPCR. Cell cytometry was assessed with MTT. Lipid content was determined by TLC. ROS were determined with 2,7-dichlorodihydrofluorescein diacetate (2 μM). LDs were stained with Nile Red (1.5 μg/mL) and visualized by confocal microscopy. Results showed that synchronized HepG2 cells displayed significant circadian rhythms in the expression of clock components (Bmal1, PER1, Rev-Erb) and choline kinase (ChoK)-like proteins, in the content of endogenous GPLs (PC and PE) and in levels of LDs (number and size and fluorescence intensity). Remarkably, when the circadian clock was perturbed by *Bmal1* KD, LDs levels and lipid enzymes were severely affected, and rhythms damped out. We found an active time-dependent control of gene expression and metabolism in proliferating HepG2 cells strongly suggesting that an intrinsic metabolic clock continues to function in proliferating liver tumor cells and its perturbation severely affected lipid metabolism. Supported by CONICET, ANPCyT-FONCYT, SECyT-UNC.

LI-P06-245
ACTIVE CALCIUM MOBILIZATION FROM A THAPSIGARGIN-SENSITIVE POOL BY FREE 32:5n-6 IN SPERMATIDS

Santiago Valtierra FX[1], Peñalva DA[1], Luquez JM[1], Aveldaño MI[1], Reyes JG[2], Oresti GM[1]
[1]INIBIBB, Dpto. BByF, UNS, Bahía Blanca, Argentina. [2]PUCV, Valparaíso, Chile.
E-mail: fsantiagov@inibibb-conicet-gob.ar

In their free form, long chain ($C_{18–22}$) polyunsaturated fatty acids (PUFA), especially 20:4*n*−6, can modify calcium homeostasis in male germ cells. These cells also contain unusual n-6 very-long-chain (VLC) PUFA (28:4, 30:5, and 32:5), in non-hydroxy (n-V) and 2-hydroxy (h-V) forms, in their membrane sphingolipids. Potential biological roles of VLCPUFA, as free fatty acids (FFA), in the physiology of male germ cells are unknown. In this study we explored the ability of n-V FFA, and their h-V counterparts, to modify the intracellular calcium homeostasis in rat spermatids. After obtaining the n-V and h-V FFA from testicular sphingomyelin, their natural source, each group was separately added to spermatids suspensions at an 8 μM concentration. The n-V FFA increased the intracellular calcium concentration ($[Ca^{2+}]_i$) in spermatids several fold more intensely than did the h-V FFA. After isolating the components of n-V and h-V mixtures by HPLC to study the effect of each VLCPUFA, free n-32:5 was found to be the most active in increasing the $[Ca^{2+}]_i$, followed by n-30:5, while h-32:5 augmented it only slightly. In addition to fatty acid-specific, the response was dose-dependent. The rates of $[Ca^{2+}]_i$ upsurge were independent of the presence of extracellular calcium. Pretreatment with thapsigargin inhibited the effect of n-32:5, suggesting that this FFA promotes the release of Ca^{2+} from intracellular calcium stores, mainly the endoplasmic reticulum. The n-V FFA did not seem to exert their effects through the G protein-coupled receptor GPR120, a putative receptor for free PUFA, as they occurred in the presence of a GPR120 inhibitor. Ceramides containing the same fatty acids did not modify $[Ca^{2+}]_i$, thus the observed $[Ca^{2+}]_i$ increases may be attributed to the FFA themselves. The possibility that they occur after VLC-FFAs are converted into other bioactive compounds remains to be investigated. Our results revealed a biological activity of VLCPUFA that suggests a physiological role for these fatty acids. As VLCPUFA-mediated Ca^{2+} rises occurred in spermatids, they may activate Ca^{2+} signaling pathways with specific functional targets in germ cells differentiation. *Supported by SGCyT UNS-PGI-UNS [24/B272 to GMO], FONCyT [PICT2017-2535 to GMO], CONICET [PIP112-201501-00711CO to MIA], and DI/PUCV to JGR.*

LI-P07-256
COMPARISON OF DIFFERENT LIPID EXTRACTION METHODS FOR ROOTS OF BARLEY

Reyna M[1], Vilchez AC[1], Peppino Margutti M[2], Villasuso AL[1]
[1]INBIAS-CONICET-UNRC, [2]CIQUIBIC-CONICET-UNC. E-mail: mreyna@exa.unrc.edu.ar

The plant lipidome is highly complex, and the composition of lipids in different tissues as well as their specific functions in plant development, growth and stress responses have yet to be fully elucidated. To do this, efficient lipid extraction protocols which deliver target compounds in solution at concentrations adequate for subsequent detection, quantitation and analysis through spectroscopic methods are required. To date, numerous methods are used to extract lipids from plant tissues. However, a comprehensive analysis of the efficiency and reproducibility of these methods to extract multiple lipid classes from roots of barley has not been undertaken. In this study, we report the comparison of three different lipid extraction procedures in order to determine the most effective lipid extraction protocol to extract lipids from root tissues of the Barley linked to a lipidomics-based ESI-MS/MS assay coupled with a statistical analysis. While particular methods were best suited to extract different lipid classes from barley roots, overall a single-step extraction method with a 24 h extraction period, which uses a mixture of chloroform, isopropanol, methanol and water, was the most efficient, reproducible and the least labor-intensive to extract a broad range of lipids for untargeted lipidomic analysis of Barley roots. In addition, the optimal protocol for barley root lipid extraction involves immediately submerging plant tissues in hot isopropanol containing 0.01% BHT right upon harvesting of fresh tissues from their respective culturing conditions, which will produce undistorted lipid profiles truly reflective of the endogenous levels of phosphatidylcholine and phosphatidic acid molecular forms (PCs and PAs). Given the critical role of PCs as predominant membrane lipids, as well as the established function of PAs as signal lipid, it is evident that strict adherence to sample pre-treatment protocol is indispensable in deriving biologically meaningful lipid profiles in plants. On the other hands, results indicate that the use of the simplified method allows improving the extraction performance of acidic lipids (PA and phosphatidylserine), which is reflected in a significant increase in the lipid content. Moreover, the data variability obtained