



52TH ANNUAL MEETING

ARGENTINE SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY

LII REUNIÓN ANUAL

*Sociedad Argentina de Investigación en Bioquímica
y Biología Molecular*

Pabellón Argentina. Universidad Nacional de Córdoba

November 7-10, 2016



- SAIB -
52th Annual Meeting
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Córdoba, República Argentina
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Cover Page:

Confocal microscopy images of Arabidopsis thaliana root are displayed in the cover. The selected roots are expressing a GFP reporter of a mitotic cyclin (CYCB1;1-GFP, green), also they are counterstained with propidium iodide (IP, red) to display the cell structure. In order to follow the progression through the cell cycle phases, the root cells were synchronized in S phase using HU, and after pictures were taken every 2 hours. This type of experiment was also used to generate RNA samples to analyze the dynamics of different gene expression during the cell cycle. Inside the circle, which shows the cell cycle phases, images of cells expressing a histone fused to the fluorescent protein VENUS and stained with IP, are displayed. Those images allow following the steps of mitosis in vivo inside the root (PL-P56: Identification of cell cycle regulators in plants, by Goldy, C; Ercoli, MF; Vena, R; Palatnik, J, Rodriguez, Ramiro E.)

Diseño de tapa: Natalia Monjes



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LI-C03

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

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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 [¹⁴C]-Acetate and [¹⁴C]-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

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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-Erba*), some phospholipid (PL) synthesizing enzyme genes and the labelling of ³²P-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

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Rat spermatogenic cell membranes contain sphingolipids with nonhydroxy and 2-hydroxy very long chain (C₂₄₋₃₂) PUFA. The biosynthesis of such fatty acids requires the expression of very long chain fatty acid elongases (*Elovl4* for > C₂₄) 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 [³H]20:4 to PUFA longer than C₂₄. 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

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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