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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 inplants, by Goldy, C; Ercoli, MF; Vena, R; Palatnik, J, Rodriguez, Ramiro E.)*

Diseño de tapa: Natalia Monjes

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 [^{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

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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-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

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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

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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

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

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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 cytochromebo_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_3strain, 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 conditionsbo_3oxidase 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*

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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*

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*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.