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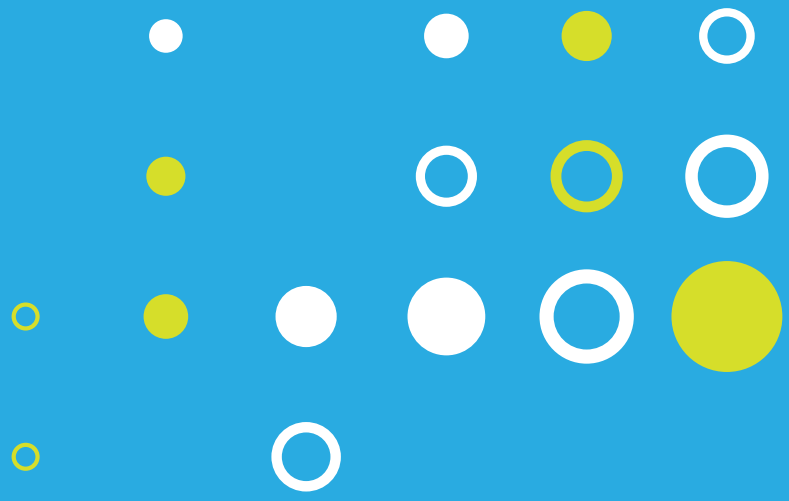

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LI-C01.

THE TYROSINE PHOSPHATASE SHP2 REGULATES THE EXPRESSION OF THE ACYL-COA SYNTHETASE ACSL4

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Acyl-CoA synthetase 4 (ACSL4) is an enzyme implicated in fatty acid metabolism which preferes arachidonic acid. cAMP-stimulation of steroidogenic cells leads to increased ACSL4 protein levels, through a pathway that requires protein tyrosine phosphatase (PTP) activity. Since NSC87877, potent inhibitor of the PTP SHP2, reduced cAMP-stimulated progesterone production (cAMP: 77±15; NSC87877+cAMP 38±9 ng/ml, $p<0.001$) in MA-10 Leydig cells, we tested whether SHP2 is involved in ACSL4 expression. For that purpose, we used a plasmid-mediated gene transfer and RNAi-mediated gene silencing approach to modify intracellular levels of SHP2 in MA-10 cells and determined ACSL4 mRNA (RT-PCR) and protein levels (Western blot) and steroid production (radioimmunoassay). Overexpression of SHP2 increased ACSL4 protein levels in cAMP-stimulated cells ($p<0.001$ vs mock). The effect could be specifically attributed to SHP2 since knock-down of this PTP by specific shRNA reduced ACSL4 mRNA and protein levels (in both cases, $p<0.01$ vs mock). Modifications in SHP2 protein levels also affected the steroidogenic capacity of MA-10 cells: overexpression or knock-down of SHP2 led to increased or decreased steroid production respectively ($p<0.01$ and $p<0.05$ vs mock respectively). In conclusion, SHP2 is at least one of the PTPs involved in the regulation of the expression of the fatty acid-metabolizing enzyme ACSL4.

LI-C02.

PHOSPHATIDYLCHOLINE: STRUCTURAL AND SIGNALING ROLE IN NEURONAL DIFFERENTIATION

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Neuritogenesis is a dynamic process, involving the extension of long protrusions called neurites, and is critically dependent on membrane biosynthesis. Phosphatidylcholine (PC) is the most abundant phospholipid in eukaryotic cells. During retinoic acid (RA) induced differentiation of neuroblastoma cells, the augmented PC synthesis is supported by the sequential activation of two enzymes of the Kennedy pathway: choline kinase (CK) and CTP:phosphocholine cytydilyltransferase alpha (CCTa). In addition, enforced CK or CCTa expression promoted neuronal differentiation even in the absence of RA.

Interestingly, we found that the addition of PC liposomes promotes neuronal differentiation by activating ERK signaling cascade, mimicking RA effects. In addition, PC-treated neurons express bIII-tubulin as a differentiation marker.

In light of these results, chemical inhibitors or siRNAs designed to specifically inhibit CK or CCTa activity, significantly abrogate the extension of neurites, attenuate ERK signaling cascade and decrease the expression of bIII tubulin.

These results allow us to propose that PC, or any of its derivative metabolites, are not only important as structural membrane components but it could also stimulate neuronal differentiation, which means a significant progress in the identification of specific signals regulating neuritogenesis.

LI-C03.

BIOSYNTHESIS OF VERY LONG CHAIN POLYENOIC FATTY ACID-CONTAINING SPHINGOLIPIDS IN GERMINAL CELLS

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In mammalian testis, sphingomyelin (SM) and ceramide (Cer) were previously shown to be rich in very long chain (VLC) polyunsaturated fatty acids (PUFA). Recent work in rat pachytene spermatocytes and round spermatids demonstrated that these sphingolipids exclusively belong to germ cells. We also recently showed that these two cell types, highly enriched in 22:5n-6-containing glycerophospholipids, express mRNA transcripts of ELOVL5 and ELOVL2, two elongases involved in the biosynthesis of C18-C22 PUFA. Because such type of PUFA need to be further elongated to produce the VLCPUFA present in rat testicular sphingolipids (C26-C32), in this work we studied the expression of ELOVL4, the enzyme expected to catalyze such elongation. During postnatal development, ELOVL4 protein expression was first detectable in the rat testis at P25, in concomitance with the timing of appearance of the first spermatocytes. In adult rat seminiferous tubules, whereas the expression of this elongase was found to be only marginal in Sertoli cells, it was significant in spermatocytes and spermatids. The use of radiolabeled precursors and the fluorescent marker NBD C6-Cer showed that isolated spermatogenic cells in culture do synthesize sphingolipids. Both results are consistent in demonstrating that spermatogenic cells are able to biosynthesize the molecular species of SM and Cer that contain VLCPUFA

LI-C04.

THE LIPID PROFILE IS A ROBUST INDICATOR OF FUNCTIONAL SENESCENCE IN THE MEDFLY *Ceratitis capitata*

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The Medfly *Ceratitis capitata* is a global orchard pest of great economic importance. Population studies in this species have been essential to answer fundamental demographic questions. We used *C. capitata* as a model to study functional senescence, combining demographic, behavioral and biochemical parameters. We previously analyzed survival curves of *C. capitata* under standard laboratory conditions (23°C, 60% room humidity), as well as changes in spontaneous locomotor activity, negative geotaxis performance and lipid profile with age. Multivariate statistical analyses showed age-dependent changes of the lipid pattern that were mostly influenced by, e.g., sterol esters, isoprenoids and fatty acid esters. To evaluate if the lipid profile could be used as a biomarker of functional state, we compared these results with those of populations maintained under a mild thermal stress of 28°C. Mean longevity of females kept at 28°C was lower than 23°C controls, while males did not present differences in their survival curves. The performance in different behavioral assays of individuals kept at 28 ° C was worse than that of individuals at 23°C. The lipid pattern of young populations at 28°C was similar to the pattern of old populations at 23°C. In conclusion, we believe that the lipid profile is a good indicator of the functional state of flies, which declines with age and under a stress condition.