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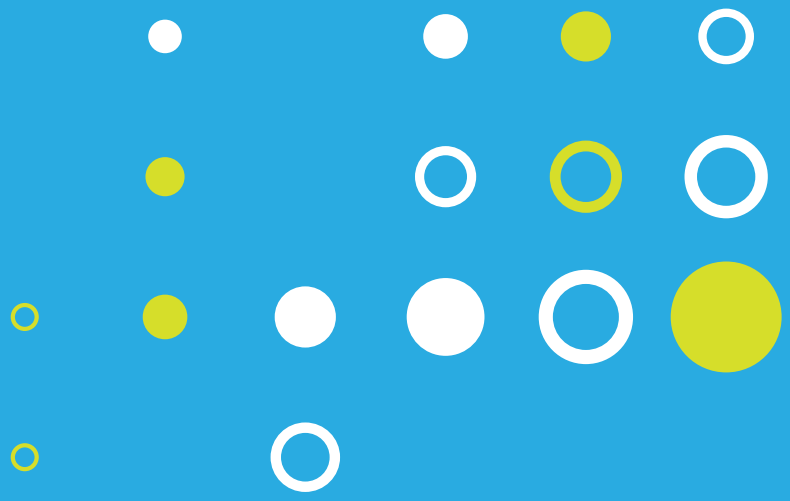

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LI-P16.
LIPID SYNTHESIS IN TESTIS: GPAT2 ACTIVITY AND EXPRESSION

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Four different isoforms of glycerol-3-phosphate acyltransferase have been described in mammalian cells. GPATs 1 and 2 are located in the mitochondria. GPAT2 is sensitive to sulfhydryl reagents (NEM), has no acyl-CoA substrate preference and was first recognized in liver from GPAT1 null mice. GPAT2 cDNA cloned from mouse testis encodes a protein of 797 aa (89 kDa) and 27% aa identity to GPAT1. Because GPAT2 mRNA expression was very high in testis, the aim of this study was to characterize the GPAT2 activity in highly purified mitochondria from wild type mouse and rat testis. The purity of the subcellular fractions was confirmed by marker enzymes and proteins. For the first time, NEM-sensitive GPAT activity was detected in purified mitochondria from wild type mouse and rat testes. We assume that this activity corresponds to the GPAT2 isoform because GPAT2 mRNA expression was more than 10 times higher in testis than in any other tissue. Although GPAT1 mRNA was 5-fold higher in liver than in testis, NEM-resistant GPAT activity in testis was 3-fold higher than in purified mitochondria from liver. In contrast, GPAT2 mRNA was 50-fold higher in testis than in liver, and, consistently, the NEM-sensitive activity in purified mitochondria was high in testis and was not detectable in liver.

LI-P17.
PHOSPHATIDYLCHOLINE BIOSYNTHESIS AND CELL GROWTH

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Phosphatidylcholine (PC) is the major phospholipid in mammalian cells. The CTP:phosphocholine cytidyltransferase (CTα) catalyze the limiting step that governs the Kennedy pathway. The understanding of the mechanisms that regulate its expression during the cell cycle will contribute to interpretate the basis of cell growth. We use CHO cells (MT58), a temperature-sensitive mutant defective in CTα, as a model. After MT58 cells are shifted to the restrictive temperature, we found that CTα is inactivated leading to cell die. The question we wish to address is why PC deficiency is essential for cell growth? To answer this question we measured changes in cell´s morphology, viability and DNA synthesis. The most important change we found was the cessation of DNA synthesis. Therefore, we investigated whether exogenous added phospholipids were able to reestablish DNA replication and if the nuclear localization of CTα is essential to induce growth.
Since the described results indicate that cell cycle progression is sensitive to PC content, we used transcriptional approaches to study the regulatory mechanism involved in Ctα expression during the cell cycle. The results indicate that this process is regulated by EF2. Since a similar mechanism regulates the expression of genes involved in DNA synthesis, we propose that both PC and DNA replication are coordinately regulated during cell growth.

LI-P18.
LIPID DROPLETS IN FRACTIONS ENRICHED IN SPERMATOCYTES, SPERMATIDS AND RESIDUAL BODIES

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In seminiferous tubules, Sertoli cells play a chief role in providing structural and functional support to spermatogenesis. Those germ cells that cannot be supported are led into apoptosis and rapidly eliminated by phagocytosis. At their luminal pole, Sertoli cells also support spermiation: as spermatids are released from the seminiferous epithelium, residual bodies are rapidly engulfed and degraded in Sertoli cells. In this work we isolated cell fractions enriched in pachytene spermatocytes, spermatids, and residual bodies to study their lipids and fatty acids. None of the germ cell fractions contained cholesterol esters, confirming that within seminiferous tubules these lipids are exclusive of Sertoli cell droplets. Glycerophospholipids rich in 22:5n-6 and sphingomyelin with 28:4n-6 and 32:5n-6 were more abundant in spermatocytes and especially in spermatids than in residual bodies. In contrast, the latter contained the highest proportion of 22:5-rich triglycerides of the three fractions. When Nile Red was used as a marker of oil droplets, spermatocytes and spermatids stained faintly and residual bodies fluoresced strongly and punctuated, indicating that 22:5-rich triglycerides were the main components of lipid droplets located within these structures. In vivo, recycling of residual body constituents including lipids and fatty acids may be one of the duties of Sertoli cells.

LI-P19.
CHOLESTEROL REMOVAL BY HUMAN APOLIPOPROTEIN A-I DEPENDS ON MEMBRANE LIPID ORGANIZATION

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We previously showed that overexpression of rat Stearoyl CoA Desaturase gene (SCD) in CHO-K1 cells induced a decrease in cholesterol (Chol) removal mediated by human apolipoproteinA-I, compared to control cells (SAIB 2006). Here we observed a differential activity of cellular acyl-CoA cholesterol acyl-transferase (ACAT) both in SCD and control cells. Furthermore, we enlarged our studies on Chol solubilization by measuring Laurdan General Polarization (GP) under two-Photon Fluorescence Microscopy. The GP value of SCD cells was similar to that of control cells, suggesting that the increase in membrane fluidity due to higher 16:1/16:0 and 18:1/18:0 ratios in phospholipids fatty acids composition, might be compensated by higher contents of membrane-ordering lipids, probably Chol. However, when Chol is removed from the membrane the GP of control cells increases, while it decreases in SCD cells. In order to interpret these results, we analyzed GP changes when Chol is removed from Giant Unilamellar Vesicles showing lipid coexistence. In this case, GP decreased when Chol was removed from more fluid domains, but it increased when Chol was removed from ordered domains. Data suggest that Chol could be solubilized preferentially from liquid-ordered domains in control cells, and from more fluid domains in SCD-cells. Results are discussed in terms of lateral phase separation.