Effect of Inhibition of TOR Pathway on Lipid Droplet Dynamics in *Saccharomyces cerevisiae*

Juliana B. Madeira¹, Bruno L Bozaquel-Morais¹, Clarissa M. Maya-Monteiro², Claudio A. Masuda¹ and Monica Montero-Lomeli¹

¹Laboratório de Biologia Molecular de leveduras, IBqM-UFRJ, RJ, Brazil  
²Laboratório de Imunofarmacologia, IOC, Fiocruz, RJ, Brazil

Lipid droplets are dynamic intracellular organelles that store neutral lipids. The signaling pathways that govern its turnover are not well understood, although it is well known that upon nitrogen depletion cells accumulate lipid droplets. In a previous work our group found that a downstream regulator of the TOR pathway, the Ser-Thr phosphatase Sit4 modulates the lipid droplet content. In a deleted SIT4 strain, the Snf1/AMPK is constitutively phosphorylated leading to a decreased content of lipid droplets. In this work we further studied the role of the TOR pathway on lipid droplet dynamics by the use of a simple fluorimetric assay. In a yeast culture two phases of lipid droplet metabolism are observed. At high glucose lipid droplets are mobilized while after six hours of growth, at the logarithmic phase, they begin to be replenished. By adding rapamycin, an inhibitor of TOR kinase, at the beginning of the second phase we observed an enhancement of the rate of accumulation of lipid droplets. Enhancement was dependent on the synthesis of triacylglycerol (TAG) but not sterol ester (SE), as the dga1/lro1 cells did not respond to rapamycin while are1/are2 mutants were insensitive. Simultaneous treatment of yeast cells with rapamycin and soraphen A, a specific inhibitor of acetyl-CoA carboxylase, blocked accumulation of lipid droplets. Based on a microarray analysis of gene expression after rapamycin treatment (Hardwick, J.S. et al., 2009) we found that genes related to glycerol, glyoxylate and TAG synthesis pathways are modulated by rapamycin. Interestingly deletion of the downstream transcription factors Gln3p and Gat1p was sufficient to inhibit accumulation of lipid droplets. In conclusion we propose that inhibition of the TOR pathway stimulates the Gln3p and Gat1p transcription factors that lead to expression of related lipid metabolism genes and further accumulation of lipid droplets in yeast.

Word Keys: lipid droplet, TOR pathway, *Saccharomyces cerevisiae*  
Supported by: CNPq and FAPERJ