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Title: FATTY ACID TRAFFICKING DURING DROSOPHILA OOGENESIS

Abstract

Lipid droplets (LDs) are ubiquitous fat storage organelles with essential roles in lipid metabolism, including storage and trafficking of fatty acids. During Stages 9-10B of oogenesis, *Drosophila* follicles accumulate hundreds of thousands of LDs containing triacylglycerol (TAG) and sterol esters. Most of the fatty acids (FAs) in these stored lipids ultimately come from lipophorin (LPP) particles circulating in the hemolymph. Previous work has led to the following working model for how these FAs are funneled into LDs: LPP particles dock on the nurse cell plasma membrane via lipophorin receptors and are broken down by extracellular lipases. The liberated FAs are then taken up via FA transporters, activated by acyl-CoA synthetases and converted to TAG by DGAT1/Midway. The long-term goal of this project is to critically test this model and identify the molecular players and regulatory steps involved. To this end, we have developed a protocol to monitor FA trafficking during oogenesis using fluorescently labeled fatty acid (FLFA). When flies are fed food supplemented with various FLFAs, FLFA accumulates in nurse cell LDs. When isolated follicles are incubated in FLFA containing media, we observe incorporation into LDs within 15 mins, providing an inroad for observing FA trafficking to nascent LDs. Preliminary evidence suggests that when flies are starved prior to FLFA feeding, FLFAs do not enrich in LDs, but accumulate in or near mitochondria. This suggests that follicles direct FLFA to different intracellular locations according to the physiological state of the animal. To better visualize the subcellular allocation of FLFA and its transfer between organelles, we developed a protocol for in-vivo centrifugation of follicles which separates major organelles by density. Combining centrifugation with supplementation of long chain and medium chain FLFA revealed that both the physiological state of the fly and FA chain length determine the intracellular distribution of the FLFA. Finally, in mutants for *midway*, no LDs form and follicles arrest at Stage 9. Using FLFA supplementation, we found that in the mutant egg chambers FLFA is still taken up, but accumulates in mitochondria, even for well-fed flies. In addition, nurse cell mitochondria take on an abnormal morphology. We hypothesize that when incoming FAs are not properly sequestered in LDs, they inappropriately accumulate in mitochondria, causing dysfunction that may contribute to the developmental arrest.