Genetics Day Poster Submission

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Title: MONITORING FATTY ACID TRAFFICKING IN FOLLICLES REVEALS A CRITICAL ROLE FOR DGAT1/MIDWAY IN PROTECTING MITOCHONDRIAL INTEGRITY

Abstract: Successful embryo development is greatly determined by the health of the oocyte. The developmental potential of oocytes can be negatively affected if their ability to uptake and utilize nutrients is perturbed during oogenesis. To help promote healthy oocyte production, the oocyte is supported by the follicle which - in Drosophila - also contains a protective epithelial layer and germ line cells (called nurse cells) that provide the oocytes with mRNAs, proteins, and nutrients, including lipids. The use of lipid during oocyte maturation has been less studied than that of other metabolites, but there is growing evidence in mammals that lipid utilization can directly affect the developmental potential of oocytes. My research is uncovering how fatty oxidation (FAO) influences mitochondrial membrane potential (MP) in nurse cells and how proper regulation of lipid utilization directly affects developmental potential. There is a detectable MP in mid-stage follicles and that MP is drastically reduced when uptake of long-chain fatty acid is blocked pharmacologically, thus presumably impairing FAO. As mid-stage follicles rapidly accumulate lipid droplets (LDs), we speculated that these LDs provide a source of fatty acids for FAO. MP is reduced in follicles mutant for ATGL, a triglyceride lipase localized to LDs, suggesting that ATGL affects the amount of FA available for energy and that LDs release FA to supply mitochondria. To determine the role of LDs in supporting mitochondria, we utilized DGAT1/midway mutants; this enzyme is responsible for the formation of triglyceride (TAG) and subsequent LD formation. midwayQX25 follicles do not form LDs in nurse cells and nurse cell mitochondria have reduced MP, supporting the notion that LDs are key for supplying FA to mitochondria and their absence reduces available FA. However, when I used follicle culturing experiments with fluorescently labeled fatty acid (FLFA), I discovered that exogenous FA has different fates in wild type (WT) and midwayQX25. In WT follicles, exogenous FA is first sequestered into LDs while in midwayQX25 follicles FA accumulates excessively in mitochondria. midwayQX25 mitochondria have an abnormal toroidal morphology which is typically indicative of excessive oxidative stress. MitoSOX staining of midwayQX25 follicles revealed their mitochondria have approximately a two-fold increase in mitochondrial ROS levels. Mitochondrial ROS in the mutant follicles can be lowered to wild-type levels by culturing follicles with the antioxidant n-acetyl-cysteine amide (NACA), a precursor for glutathione synthesis; in turn, MP returned to WT levels. In addition, we have shown that mitochondrial ROS levels in midwayQX25 follicles are also reduced by blocking CPT1 activity. We propose a model for FA trafficking in WT conditions: exogenous FA is taken up from lipoproteins circulating in the hemolymph. FA is converted into TAG and stored in that form in LDs where lipase(s) can release FA that can be shuttled to mitochondria for FAO. midwayQX25 still take up FA but cannot properly sequester FA as TAG. FA is excessively shuttled to mitochondria, leading

to increased mitochondrial ROS, toroid formation, and decreased MP. We speculate that these insults to mitochondria may contribute to the developmental arrest seen in midwayQX25 follicles. These data support the notion that proper regulation of FA trafficking and utilization directly affects the developmental potential of oocytes