Analysis of the distribution of lipid droplets, and characterization of specialised cortex glial cells in Drosophila melanogaster

Main points of the Ph.D. thesis

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Introduction

Lipid droplets (LDs) are common organelles of the majority of eukaryotic cell types. These structures are generally regarded as the cellular storage sites of lipids, including neutral lipids (sterols, triacylglycerols) and a repository for the precursors of phospholipids, the building blocks of cellular membranes [Farese and Walther 2009, Thiele and Spandl 2008]. During the last decades considerable data has emerged in the literature proving that LDs serve not merely as passive stores of excess fat and other lipid substances, but they simultaneously participate in many different cellular processes, such as intracellular protein and phospholipid metabolism during cell division [Barba et al. 1997], the replication of the Hepatitis C virus [Miyanari et al. 2007], and proteasomal protein degradation [Ohsaki et al. 2006]. A hallmark of LD research was the discovery that LDs function as a protein storage depot in cells. The presence of over 150 various proteins has been linked to LDs [Cermelli et al. 2006, Beller et al. 2006]. Moreover, LDs are involved in intracellular protein metabolism [Miyanari et al. 2007, Cermelli et al. 2006]. There is ample evidence proving that LDs play a crucial role in the pathophysiology of certain human diseases, such as obesity, metabolic syndrome, fatty liver syndrome and atherosclerosis [Guzton et al. 1989, Lang et al. 1970, Reddy and Sambasiva Rao 2006, Cohen et al. 2011, Greenberg et al. 2012]. LD cell biology and physiology have been extensively studied in liver cells and in the adipose tissue of mammals [Zimmermann et al. 2004, Wake et al. 1974, Ashworth et al. 1960]. Although the nervous system contains the highest relative amount of lipids, the spatiotemporal distribution and physiological function of LDs in the brain remain largely unknown. The Drosophila melanogaster is an excellent genetic model for higher animals, since in contrast with mammalian systems, gene redundancy is minimal in flies, allowing scientists to analyze in vivo gene functions. The Drosophila also has a short life cycle, a wide variety of available genetic tools, and mutants and RNAi lines have been systematically generated [Chang and Neufeld 2010]. The most powerful genetic tool in Drosophila is the Gal4-

UAS dual transgenic system, where Gal4 is a transcription factor that selectively binds to the Upstream Activating Sequences (UAS) and enhances the expression of the downstream DNA sequences [Duffy 2002]. This allows a variety of transgenic techniques such as targeted gene expression modification (overexpression or RNA silencing) by expressing the Gal4 under the control of tissue-specific promoters and fusing transgenes or ds RNA sequences after the UAS. Moreover, while a large portion of the Drosophila neurodegeneration mutants (bubblegum, swiss cheese, loechrig, ApoD, frataxin, sicily) [Navarro et al. 2010, Kretzschmar and Pflugfelder 2002, Tschape et al. 2002] affect lipid metabolism and disturb LD homeostasis, neither the cellular, nor the spatio-temporal distribution of LDs has been described to date in Drosophila. In this paper, we used the brain of the fruitfly to study lipid droplet anatomy in the larval nervous system.

Materials and Methods

1. Fruitfly stocks and genetics
2. Generation of flip-out clones
3. Production of the Dfabp antisera
4. Western blotting
5. Histology, immunostainings and imaging
6. Oil Red O staining
7. Semithin sections
8. Routine electron microscopy and HRP cytochemistry
9. Freeze substitution and LR White embedding
10. Post-embedding silver-intensified immunogold labeling of freeze substituted LR White-embedded sections
11. Quantification and data anaylsis

Results, thesis

- LDs were present throughout the entire brain but were preferentially enriched in the medial part of the central brain. We found LDs to be organized in large clusters in resin sections. In the electron microscope (EM), LDs were found exclusively in the glial cells. Interestingly, we determined that 65.5% of LDs (310 of 473 LDs) were found in the closest vicinity (<10 μm) of neuroblasts, located in the outer layers of the brain cortex.

- We found that while LDs are absent in the embryonic stage and in the first instar larvae, the density of LDs gradually increases from the beginning of the second larval stage until the end of the wandering period of the last larval stage, when it reaches its highest value, after which it decreases gradually until the end of the pupal stage.

- During clonal analysis we concluded that glial subtypes contained different amounts of LDs but the cortex glial cells located in the superficial region of the brain cortex accumulated significantly more LDs in comparison to the other types. These lipid accumulating cortex glial cells had characteristic processes encapsulating unlabeled large areas, possibly cell bodies of neurons.

- To verify our observations at the EM level, we labeled cortex glial membranes with a membrane- targeted HRP, which can be visualised after the DAB reaction. As expected, we found that these superficial cortex glial cells insulated mitotic neuroblasts. The cells accumulated high amounts of LDs in their perinuclear region, had invaginated dense heterochromatic nucleus and often also contained a high amount of glycogen. The cells were tightly attached to subperineurial cells. Another interesting finding that EM

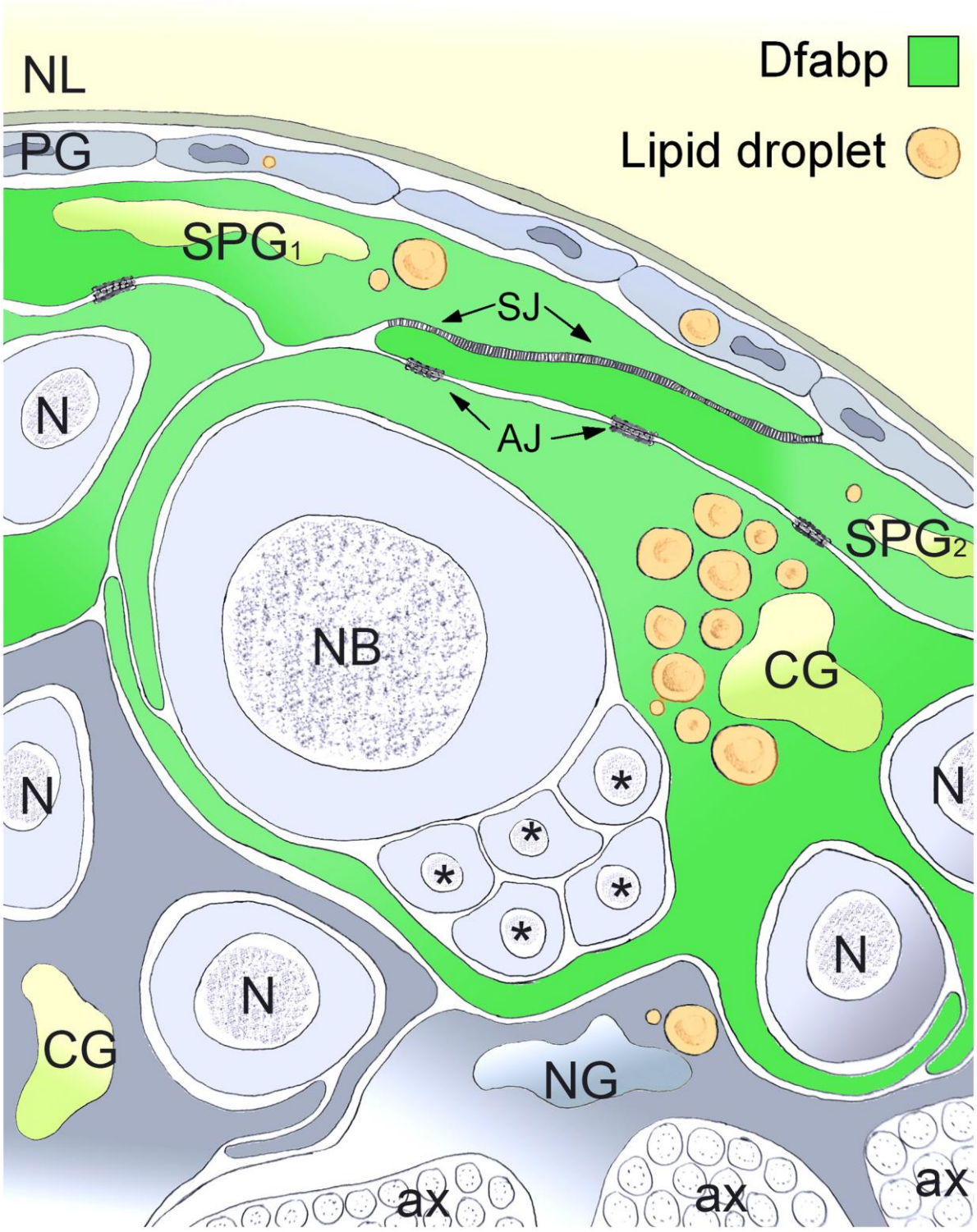
showed was that while neighbouring SPGs establish septate junctions, SPG and cortex glial cells were connected to each other through adherens junctions.

- Next, we wanted to find molecular markers that would be specific to LD accumulating superficial cortex glial cells. With this objective in mind, we performed a literature based screen for lipid metabolism-related genes expressed in the Drosophila CNS. We found that the Drosophila fatty acid binding protein (dfabp, CG6783), an orthologue of the mammalian fatty acid binding protein 7 (FABP7/brain lipid binding protein, BLBP) is preferentially expressed in glial cells of the embryonic nervous system. We were interested in whether or not this protein is indeed expressed in third instar larval glial cells. To test this, we raised polyclonal antisera against the third exon of dfabp. The Dfabp antibody showed a strong staining in a layer at the surface of the brain and also a deeper thin network likely to correspond to a subset of glial cells. The pattern of the immunoreactivity seemed to surround clusters of unlabeled cell bodies.

- We wanted to confirm our light microscopic observations at the EM level as well. Accordingly, we carried out post-embedding immunogold labeling to determine the precise cellular and subcellular localisation of Dfabp. In the EM, we found cortex and subperineurial cells to be strongly immunoreactive for Dfabp, while perineurial cells, neuroblasts, and neurons were always immunonegative. In glial cells, Dfabp labeling was detected over the cytosol, nucleoplasm, and glycogen areas. No labeling was observed over mitochondria, ER, or LDs. Superficial cortex glial cells, containing numerous LDs, were always strongly immunopositive for Dfabp. We concluded that our Dfabp antibody selectively labels the subperineurial and the lipid accumulating superficial cortical glial cells in the third instar larval brain.

Discussion

LDs are common organelles of eukaryotic cells, participating in a variety of cellular processes. Although lipids are highly enriched in the nervous system, the spatio-temporal distribution and physiological function of LDs in the developing brain is poorly understood. In this work, we demonstrated the highly specific spatio-temporal distribution of LDs in the nervous system of Drosophila. Key findings of our work are summarized in the artwork of Barti Benjamin. We showed that LDs are present throughout the entire larval brain but are preferentially enriched in the medial part of the central body. LDs are concentrated in large clusters in the perinuclear region of glial cells but are not present at all in neurons. In this brain area the majority of LDs is found in the closest vicinity of neuroblasts. The unique spatial segregation of LDs between neurons and glial cells, and the time-course changes in the amount of LDs together suggest that LDs may serve specific functions during brain development through a novel, yet unknown way of neuron-glia interaction. The amount of LDs start to decrease in the middle of the pupal stage, just after the mitotic activity of neuroblasts terminates and newborn neurons start to develop processes. The dynamics of LDs raise the possibility that during development, free fatty acids mobilised from LDs may promote distinct cellular processes, such as cell division and cell membrane expansion, two important features of the developing brain [D'Alessandro et al. 2010, Pfenninger 2009]. Another explanation for the function of LDs could be that they provide energy during brain development. However, given the amount of glycogen accumulated by neurons and glia, it is unlikely that LDs would function as energy sources. In rats it has been shown that the brain does not use fatty acids for energy production [Yang et al. 1987]. At the same time, membrane building blocks can only be synthesized from lipids mobilised from the LD pool and not from the glycogen



Schematic illustration of the relative distribution of lipid droplets between glial cell types and the localization of Dfabp. LDs are concentrated in large clusters in the perinuclear region of glial cells but are not present at all in neurons (N). Specialized superficial cortex glial cells (CG) insulating neuroblasts (NB) and their daughter cells (asterisks) accumulating the highest amount of LDs. Neighboring subperineurial cells (SPG) establish septate junctions (SJ), while SPG and superficial cortex glial cells are connected to each other through adherens junctions (AJ). The Drosophila fatty acid binding protein (Dfabp) is expressed in LD accumulating superficial cortex glial cells and subperineurial (SPG) cells, and is localized in the cytosol and in the nucleus. NL: neural lamella, PG: perineural glia, NG: neuropil glia, ax: axon.

stores. In addition, we found that the Drosophila fatty acid binding protein (Dfabp, CG6783) is expressed in superficial cortex glial cells and subperineurial (SPG) cells, and is localized in the cytosol and in the nucleus. Nuclear localisation of the fatty acid binding proteins was reported previously in mammals [Feng et al. 1995, Liu et al. 2010]. Since cytosolic fatty acid binding proteins are known to be cellular lipid carriers [Schaap et al. 1999], the presence of Dfabp in SPGs and superficial cortex glial cells raises the possibility that Dfabp transfer fatty acids from the hemolymph into glial LDs. The mammalian orthologue of dfabp, the brain lipid binding protein (BLBP/FABP7) is expressed in radial glial cells in the developing brain and in astrocytes in the mature nervous system [Feng et al. 1995, Gerstner et al. 2012, Matsumata et al. 2012]. FABP7 KO mice have a decreased number of astrocytes, neural stem cells and early progenitor cells in the developing brain [Watanabe et al. 2007]. Interestingly, FABP7 mice also exhibit increased fear memory and enhanced anxiety without any visible histological abnormalities [Owada et al. 2006]. It should be noted that investigating the function of FABPs in mammals is problematic, because three fatty acid binding proteins (FABP3, FABP5, FABP7) are present in the mammalian nervous system with partially overlapping expression pattern [Liu et al. 2010], and in KO animals other FABPs may compensate for the loss of the gene of interest. For that reason, Drosophila offers an excellent opportunity to study the function of dfabp, since there is no other gene in the genome that can compensate for the loss of dfabp. To the best of our knowledge this is the first study that presents detailed anatomical data about LDs in the Drosophila nervous system. We showed that LDs are transient organelles of Drosophila glial cells and the majority of these LDs are localised in specialized superficial cortex glial cells that express the Drosophila fatty acid binding protein Dfabp.

Publications

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