Control of Fat Storage by a Drosophila PAT Domain Protein

Sebastian Grönke,' Mathias Beller,' Sonja Fellert, Hariharasubramanian Ramakrishnan, Herbert Jäckle, and Ronald P. Kühlneina
Max-Planck-Institut für Biophysikalische Chemie Abteilung Molekulare Entwicklungsbiologie Am Fassberg 11
37077 Göttingen
Germany

Summary

In Drosophila, the masses and sheets of adipose tissue that are distributed throughout the fly are collectively called the fat body. Like mammalian adipocytes, insect fat body cells provide the major energy reserve of the animal organism. Both cell types accumulate triacylglycerols (TAG) in intracellular lipid droplets; this finding suggests that the strategy of energy storage as well as the machinery and the control to achieve fat storage might be evolutionarily conserved. Studies addressing the control of lipid-based energy homeostasis of mammals identified proteins of the PAT domain family, such as Perilipin [1], which reside on lipid droplets [2]. Perilipin knockout mice are lean and resistant to diet-induced obesity [3, 4]. Conversely, Perilipin expression in preadipocyte tissue culture increases lipid storage by reducing the rate of TAG hydrolysis [5, 6]. Factors that mediate corresponding processes in invertebrates are still unknown. We examined the function of Lsd2, one of only two PAT domain-encoding genes in the Drosophila genome. Lsd2 acts in a Perilipin-like manner, suggesting that components regulating homeostasis of lipid-based energy storage at the lipid droplet membrane are evolutionarily conserved.

Results and Discussion

Comparison of the two Drosophila PAT domain-encoding genes, Lsdp1 and Lsd2 [7], with genes present in Anopheles gambiae, Bombyx mori, and Dictyostelium discoideum suggest that insect genomes encode only two PAT domain proteins (Figure 1A). In both Drosophila and Dictyostelium, they are associated with intracellular lipid droplets [8]. However, they cannot be directly homologized to any of the vertebrate family members. In order to demonstrate a possible conserved function, we characterized the Lsd2 gene and asked whether its product participates in the regulation of lipid storage in the fly, as observed with mammalian Perilipin.

Generation of Lsd2 Mutants
Lsd2 is located at the cytogenetic map position 13A9-10 on the Drosophila X chromosome. The transcript contains four exons adding up to 2.2 kb in length [9]. (Figure 1B). The mutant Lsd251 [10, 11] carries a P(SUPor-P) insertion in the Lsd2 5' untranslated leader (Figure 1B) and 3' untranslated leader (Figure 1B) as well as two Lsd2 deletion mutants (Lsd2rev/KG00149, Lsd2rev/KG00149) by a conventional P element mobilization scheme [12] involving the Lsd251 [10, 11] allele (Figure 1B). The precise excision allele Lsd2revKG00149 contains the wild-type gene, whereas the deletion mutants lack Lsd2 sequences from position –34 to +654 and +34 to +970 relative to the putative translational start codon. Western blot analysis with antibodies directed against the bacterially produced protein (α-LSD2 antibodies) reveals a doublet of bands with apparent molecular weights of 46 kDa and 44 kDa (Lsd2H and Lsd2L) in both wild-type and revertant Lsd2revKG00149 flies, whereas no Lsd2 bands could be detected in protein extracts of the Lsd2 mutants (see below). This indicates that these alleles are Lsd2 protein null mutants and that the α-LSD2 antibodies are specific.

Expression Profile and Localization of Lsd2
Lsd2 is expressed during all stages of the Drosophila life cycle, and transcripts accumulate in specific spatiotemporal patterns. Northern Blot analysis demonstrates a strong enrichment of the 2.4 kb Lsd2 mRNA in early embryos (Figure 2A), and this enrichment reflects a maternal contribution, as also visualized by whole-mount in situ hybridization of syncytial blastoderm-staged embryos (Figure 2B). Maternal Lsd2 mRNA becomes subsequently degraded, except in germline precursor cells, where the transcripts are enriched until midembryonic stages (Figures 2C and 2D). There is transient Lsd2 expression in the amnioserosa (Figure 2E) and continuous expression in the developing fat body (Figures 2F and 2G) and the anterior midgut (Figure 2G), two tissues known to function in lipid storage and nutrient lipid resorption, respectively. During the first and second larval stages, Lsd2 is only moderately expressed. In third instar larvae, the gene is strongly expressed in the fat body, the major TAG storage tissue (Figure 2H and the Supplemental Data available with this article online).

In order to visualize the intracellular localization of Lsd2 in vivo, we targeted expression of an LSD2-EGFP fusion protein to the third instar larval fat body by using the Ga4/UAS system [13] in conjunction with a fat body-specific Ga4 driver (FB-Ga4). In living fat body cells of such individuals, LSD2-EGFP is associated with vesicular structures of various sizes (Figure 3A). Purification of their fat bodies' intracellular vesicles by density gradient fractionation results in the detection of LSD2-EGFP on lipid droplet surfaces, as identified by Nile red staining (Figure 3B). Moreover, Western blot analysis of density-fractionated fat body homogenates of third instar larva shows LSD2 enrichment in the lipid droplet fraction (Figure 3C). The spatiotemporal expression patterns and the intracellular localization of Lsd2 are therefore consistent with the proposal that the protein plays a regulatory role in global TAG storage by acting at the level of lipid droplets.
reverted the leanness of Lsd2KG00149 domain family members encoded by insect genomes, are contained Perilipin. This suggests that LSD2 operates in a Perilipin-

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is reduced by 34.5%, 28%, and 37.2%, respectively.

Loss-of-Function Lsd2 Mutants Are Lean

Lsd2 mutant flies contain significantly less TAG levels than controls (Figure 4A). For simplicity, we refer to those individuals as lean and to those exceeding the TAG levels of controls as obese. As compared to freshly hatched Lsd2revKG00149 male individuals that carry a functional Lsd2 allele (lane 1), the TAG content of Lsd2KG00149 (lane 2), Lsd240 (lane 3), and Lsd2revKG00149 (lane 4) mutants is reduced by 34.5%, 28%, and 37.2%, respectively. In order to unambiguously establish whether the TAG reduction is caused by the loss of LSD2 function in the fat body, we expressed a cDNA-based Lsd2 transgene the organismal control of lipid storage howestasis. The LSD2 ORF

Figure 1. PAT Domain Family and Molecular Characteristics of the Drosophila Lsd2 Gene

(A) Sequence similarity tree based on PAT domain amino acid sequences of selected vertebrate and invertebrate PAT domain-encoding genes. Representatives of Lsdp1 and Lsd2, the two PAT domain family members encoded by insect genomes, are contained in the box.

(B) Lsd2 gene structure and gene locus organization of P element integration mutant Lsd2KG00149 and deletion mutants Lsd240 and Lsd2revKG00149 compared to precise excision revertant Lsd2revKG00149. The LSD2 ORF is indicated in light gray, and PAT domain localization is indicated in dark gray.

For Experimental Procedures, see the Supplemental Data available with this article online.

Overexpression of Lsd2 Causes Obesity

In order to test whether LSD2 activity is capable of modulating the TAG level of otherwise wild-type flies, we overexpressed Lsd2 in the fat body. Western blots with proteins extracted from freshly hatched male flies tested with α-LSD2-specific antiserum show gradually increased levels of UAS-Lsd2 transgene-dependent LSD2 activity in the fat body, and these increased levels result in increasingly severe obesity phenotypes (Figure 4A). Flies moderately overexpressing Lsd2 elevate organismal TAG storage by 28% (FB-Gal4; lane 7), whereas strong Lsd2 overexpression causes a TAG storage increase by 48.5% (Adh-Gal4; lane 8) compared to control individuals bearing the noninduced UAS-Lsd2 transgene (lane 6). These data demonstrate that modulation of LSD2 levels is sufficient to adjust TAG storage in ad libitum fed flies. The obese FB-Gal4-UAS-Lsd2 flies are more starvation resistant than control flies, whereas the lean Lsd2revKG00149 mutant flies are starvation sensitive (Figure 4B). Starvation resistance of Lsd2-overexpressing flies is accompanied by a delayed but complete pre-mortal depletion of the TAG stores (data not shown). Collectively, these results indicate that Lsd2 activity can adjust TAG storage at an organismal level at times when food is accessible to ensure extended survival when food supply is limiting.

Our results provide evidence that Lsd2 of Drosophila is an essential component of the genetic circuitry that controls energy homeostasis at the level of fat storage. The finding that varying the amount of LSD2 causes a dosage-dependent increase of TAG storage, whereas the lack of LSD2 results in lean flies, is reminiscent of results obtained with the vertebrate PAT domain protein Perilipin. This suggests that LSD2 operates in a Perilipin-like manner by modulating the rate of lipolysis. The results also suggest that PAT domain proteins, which are found in higher eukaryotes as diverse as human, fly, and the slime mold Dictyostelium, share an ancestral function in the organismal control of lipid storage homeostasis. The Drosophila flies with Lsd2 lack-of-function and gain-of-function genotypes introduced here therefore represent a genetically accessible model system to identify the components and mechanisms underlying the phenomenon of energy homeostasis in order to address questions concerning energy storage disorders.

Supplemental Data

Supplemental Data including the Experimental Procedures and a supplemental figure are available at http://images.cellpress.com/supmat/supmatin.htm.

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Figure 2. Developmental Expression of the Lsd2 Gene

(A) Developmental Northern blot analysis detects a single 2.4 kbp Lsd2 mRNA present throughout the flies’ life cycle with high abundance in early embryos, third instar larvae, and adults of both genders.

(B–H) Tissue distribution of (B–G) embryonic and (H) third instar larval Lsd2 gene expression shown by RNA in situ hybridization. Ubiquitously distributed maternal Lsd2 mRNA at the (B) early blastoderm stage gets degraded at the (C) cellular blastoderm stage, with the exception of the germ cells, which express Lsd2 during germ band extension (D). During germ band retraction (E and F), Lsd2 is temporarily expressed in the amnioserosa and comes up in the developing fat body, where it is active during late embryonic stages (G), at which time the first midgut compartment expresses Lsd2 as well. (H) Part of a third instar larval salivary gland (sg) with attached fat body tissue (fb) exemplifies fat body-specific Lsd2 expression. (B–D) Lateral and (E–G) dorsal views of embryos; the arrows highlight expression in embryonic and larval fat body tissue.

For Experimental Procedures, see the Supplemental Data.


Figure 3. Lipid Droplet Association of LSD2 Protein

(A) Confocal microscopic image of a third instar larval fat body cell expressing an LSD2-EFGP fusion protein showing association of the fusion protein with intracellular vesicles.

(B) Epifluorescence microscopic image of an isolated lipid droplet after density gradient fractionation of larval fat body tissue described in (A). The LSD2-EGFP fusion protein is tightly associated with the lipid droplet surface. Nile red staining confirms the identity of the vesicle as a lipid storage droplet.

(C) Western blot analysis of endogenous LSD2 intracellular localization in wild-type third instar larval fat body cell homogenates fractionated by density gradient centrifugation. The upper panel shows the protein concentration of density fractions. The lower panels show strong enrichment of LSD2 in a lipid droplet and low-density midzone fractions detected by α-LSD2 antisera. Cytoplasmic fractions are identified by the presence of eIF4A. Note: LSD2 is represented by LSD2H (46 kDa) and LSD2L (44 kDa); protein loading is adjusted in Western blot samples. The weak signal in eIF4A lane 2 originates from incomplete stripping of the LSD2H signal.

For Experimental Procedures, see the Supplemental Data.

Figure 4. Correlation of LSD2 Protein Amount, TAG Content, and Survival Time under Starvation in Male Flies with Lsd2 Lack-of-Function and Gain-of-Function

(A) Total TAG content (in μg TAG/mg total protein ± standard deviation) of flies correlated to their LSD2 content shown by Western blot analysis with α-LSD2 antisera (α-Tubulin for normalization). Lane 1: normal TAG content (1014 ± 28) of Lsd2−/− flies expressing endogenous LSD2 level. Lanes 2–4: Lsd2 protein null mutants are lean (lane 2: Lsd2Δ [663 ± 67]; lane 3: Lsd2Δ [730 ± 105]; lane 4: Lsd2Δ [636 ± 46]). Lane 5: reversion of Lsd2Δ leaness by FB-Gal4:UAS-Lsd2 expression (1170 ± 67). Lanes 6–8: increasing obesity and LSD2 abundance of noninduced (lane 6: 1212 ± 45), FB-Gal4-induced (lane 7: 1402 ± 89) UAS-Lsd2 transgenic flies. Note: UAS-Lsd2 induction provides LSD2H only.

(B) Starvation survival profiles of male flies parallel Lsd2-dependent TAG storage levels. Lean Lsd2Δ mutant flies (solid line) have a reduced median lifespan under water-only starvation, while Lsd2-overexpressing flies (stippled line) are starvation resistant compared to control flies (noninduced UAS-Lsd2 transgene; dashed line). For Experimental Procedures, see the Supplemental Data.