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Control of Fat Storage by a *Drosophila* PAT Domain Protein

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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 $Lsd2^{KG00149}$ carries a P{SUPor-P} insertion in the *Lsd2* 5′ untranslated leader (Figure 1B) [10, 11]. We generated a precise excision revertant ($Lsd2^{revKG00149}$) as well as two *Lsd2* deletion mutants ($Lsd2^{51}$, $Lsd2^{40}$) by a conventional P element mobilization scheme [12] involving the $Lsd2^{KG00149}$ allele (Figure 1B). The precise excision allele $Lsd2^{revKG00149}$ 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 $Lsd2^{revKG0014}$ 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 up 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 Gal4/UAS system [13] in conjunction with a fat body-specific Gal4 driver (FB-Gal4). 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.

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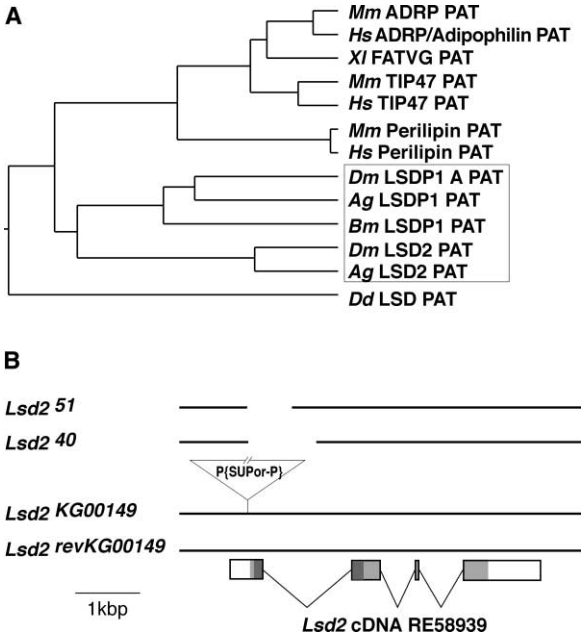

Figure 1. PAT Domain Family and Molecular Characteristics of the *Drosophila Lsd2* Gene

(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 $Lsd2^{KG00149}$ and deletion mutants $Lsd2^{40}$ and $Lsd2^{51}$ compared to precise excision revertant $Lsd2^{revKG00149}$. 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.

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 $Lsd2^{revKG00149}$ male individuals that carry a functional *Lsd2* allele (lane 1), the TAG content of $Lsd2^{51}$ (lane 2), $Lsd2^{40}$ (lane 3), and $Lsd2^{KG00149}$ (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 (UAS-*Lsd2*) in response to the FB-Gal4 driver in *Lsd2* mutant individuals. Expression of *Lsd2* in the fat body reverted the leanness of $Lsd2^{KG00149}$ mutant flies (Figure 4A, lane 5), indicating that loss of LSD2 activity is the cause of the mutant phenotype. Thus, LSD2 is an essential component in the regulation of lipid storage in the fly fat body.

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 $Lsd2^{40}$ mutant flies are starvation sensitive (Figure 4B). Starvation resistance of *Lsd2*-overexpressing flies is accompanied by a delayed but complete premortal 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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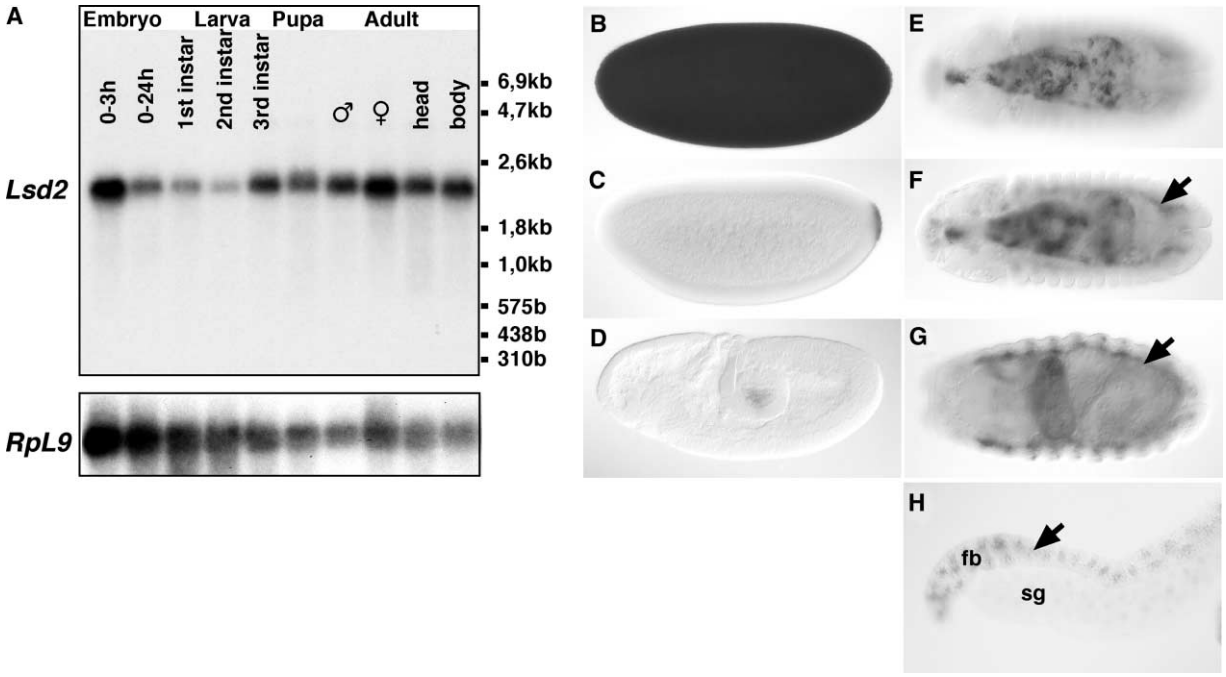

Figure 2. Developmental Expression of the *Lsd2* Gene

(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 gender.

(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.

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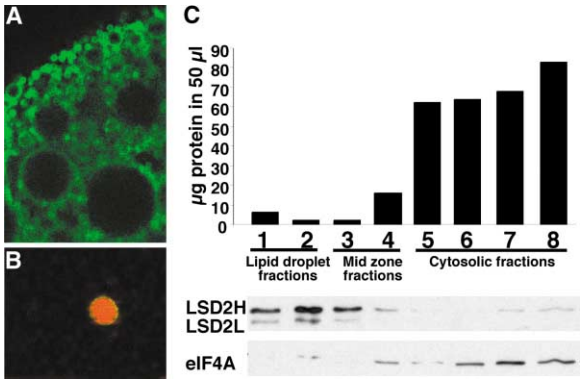

Figure 3. Lipid Droplet Association of LSD2 Protein

(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) Epiflourescence 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 antiserum. 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.

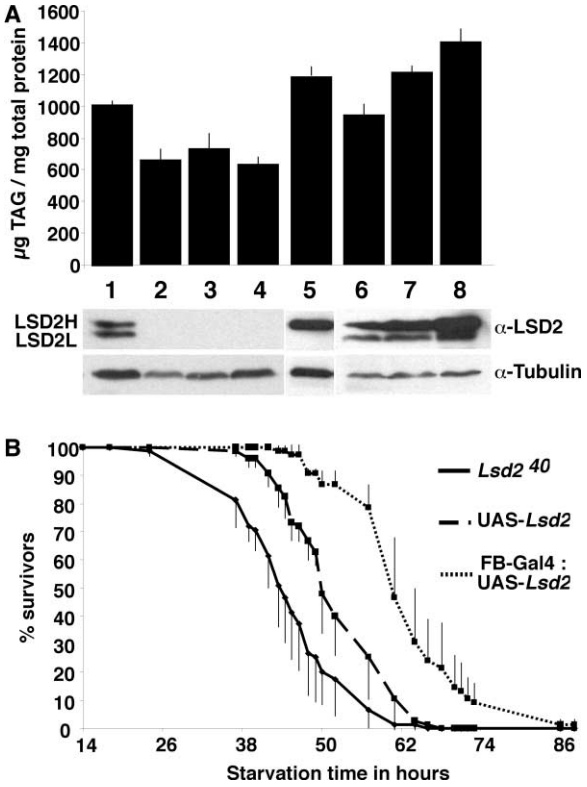

Figure 4. Correlation of LSD2 Protein Amount, TAG Content, and Survival Time under Starvation in Male Flies with *Lsd2* Lack-of-Function and Gain-of-Function

(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 antiserum (α-Tubulin for normalization). Lane 1: normal TAG content (1014 ± 28) of $Lsd2^{revKG00149}$ flies expressing endogenous LSD2 level. Lanes 2–4: *Lsd2* protein null mutants are lean (lane 2: $Lsd2^{51}$ [663 ± 67]; lane 3: $Lsd2^{40}$ [730 ± 105]; lane 4: $Lsd2^{KG00149}$ [636 ± 46]). Lane 5: reversion of $Lsd2^{KG00149}$ leanness by FB-Gal4:UAS-*Lsd2* expression (1170 ± 67). Lanes 6–8: increasing obesity and LSD2 abundance of noninduced (lane 6: [944 ± 70]), FB-Gal4-induced (lane 7: [1212 ± 45]), and *Adh*-Gal4-induced (lane 8: [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 $Lsd2^{40}$ 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.