

Metabolomic Analysis Reveals That the *Drosophila melanogaster* Gene *lysine* Influences Diverse Aspects of Metabolism

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ABSTRACT The fruit fly *Drosophila melanogaster* has emerged as a powerful model for investigating the molecular mechanisms that regulate animal metabolism. However, a major limitation of these studies is that many metabolic assays are tedious, dedicated to analyzing a single molecule, and rely on indirect measurements. As a result, *Drosophila* geneticists commonly use candidate gene approaches, which, while important, bias studies toward known metabolic regulators. In an effort to expand the scope of *Drosophila* metabolic studies, we used the classic mutant *lysine* (*lys*) to demonstrate how a modern metabolomics approach can be used to conduct forward genetic studies. Using an inexpensive and well-established gas chromatography-mass spectrometry-based method, we genetically mapped and molecularly characterized *lys* by using free lysine levels as a phenotypic readout. Our efforts revealed that *lys* encodes the *Drosophila* homolog of Lysine Ketoglutarate Reductase/Saccharopine Dehydrogenase, which is required for the enzymatic degradation of lysine. Furthermore, this approach also allowed us to simultaneously survey a large swathe of intermediate metabolism, thus demonstrating that *Drosophila* lysine catabolism is complex and capable of influencing seemingly unrelated metabolic pathways. Overall, our study highlights how a combination of *Drosophila* forward genetics and metabolomics can be used for unbiased studies of animal metabolism, and demonstrates that a single enzymatic step is intricately connected to diverse aspects of metabolism.

KEYWORDS *Drosophila*; metabolomics; lysine; LKRSDH; familial hyperlysinemia

Our modern understanding of *Drosophila* metabolism is due, in part, to genetic screens conducted during the early 20th century. Many of the classic mutations isolated by Morgan and his colleagues—such as *vermillion*, *rudimentary*, *cinnabar*, *ebony*, *rosy*, and *Henna*—disrupt key metabolic enzymes (Lindsley and Zimm 1992). Subsequent analysis of these mutants helped establish the field of biochemical genetics and provided key insights regarding the *in vivo* regulation of intermediate metabolism. In contrast to these classic forward genetic studies, modern analyses of *Drosophila* metabolism primarily rely on reverse genetic approaches, which, while important, inevitably bias our understanding of animal

metabolism toward gene families with known roles in human metabolic disease.

The importance of using forward genetics to study fly metabolism is exemplified by the *Drosophila* gene *adipose* (*adp*), which was initially identified in the 1950s as a regulator of the starvation response and triglyceride storage (Doane 1960; Teague *et al.* 1986). Forty years later, the molecular cloning of *adp* uncovered a novel, highly conserved gene that regulates triglyceride metabolism in organisms ranging from *Caenorhabditis elegans* to humans (Hader *et al.* 2003; Suh *et al.* 2007). Similarly, forward genetic screens have uncovered dozens of conserved genes that influence triglyceride and carbohydrate metabolism in *Drosophila* larvae (Pospisilik *et al.* 2010; Reis *et al.* 2010; Ugrankar *et al.* 2015; Song *et al.* 2017). Despite such successes, the use of forward genetics in metabolic research, and especially in studies of central carbon metabolism, is limited due to the tedious nature of using metabolites as the primary phenotypic readout. However, recent advances

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in metabolomics have simplified the extraction and measurement of small molecule metabolites from *Drosophila* tissues (Cox *et al.* 2017), making forward genetic studies of intermediary metabolism a realistic possibility. To demonstrate the feasibility of using metabolomics to conduct phenotype-driven analysis, we reexamined the classic *Drosophila* mutation *lys*¹ using gas chromatography-mass spectrometry (GC-MS).

E. H. Grell serendipitously discovered *lys*¹ as a background mutation present within Ed Lewis' stock collection (Grell 1961). Yet, even though the *lys*¹ mutation induces highly elevated lysine levels, *lys*¹ mutants fail to display visible phenotypes under normal growth conditions (Grell 1961). As a result, this gene has not been studied in over 50 years. Here, we genetically mapped and molecularly characterized *lys*¹ by using a GC-MS-based method to directly measure lysine abundance. Our efforts revealed that *lys*¹ disrupts the *Drosophila* ortholog of Lysine Ketoglutarate Reductase/Saccharopine Dehydrogenase (*LKRSDH*), which encodes the first enzyme involved in lysine catabolism and is mutated in humans with familial hyperlysinemia (Markovitz *et al.* 1984; Cakouros *et al.* 2008). We then used the same GC-MS-based method to conduct a targeted metabolomic analysis of *lys*¹, thereby allowing us to rapidly characterize the metabolic phenotype of these mutants. This analysis revealed that the *lys*¹ mutant exhibits a metabolic profile reminiscent of patients with familial hyperlysinemia, suggesting that flies and humans catabolize lysine using similar metabolic mechanisms. Furthermore, our metabolomics approach uncovers novel relationships between lysine and other compounds involved in amino acid and carbohydrate metabolism, emphasizing that even a relatively simple enzymatic step can influence seemingly unrelated metabolic processes. Overall, our study demonstrates how metabolomics can simplify forward genetic studies of *Drosophila* intermediate metabolism, and raises the possibility that a similar method could be used in future genetic screens.

Materials and Methods

Drosophila husbandry and strain creation

Fly stocks were maintained at 25° on Bloomington *Drosophila* Stock Center (BDSC) food. Unless noted, all mutations and transgenes were studied in a *w*¹¹¹⁸ background. The chromosome containing *lys*¹ was isolated from BDSC stock #692 (*lys*¹ *rc*¹; *ss*¹) by crossing mutant males with *w*¹¹¹⁸; *In*(2*LR*) *Gla*, *wg*^{*Gla*-1}/CyO, *P*{*GAL4*-*twi*.*G*}2.2, *P*{*UAS*-2*xEGFP*}*AH2*.2 (BDSC stock #6662) virgin females. F1 male progeny of the genotype *w*¹¹¹⁸; *lys*¹ *rc*¹/CyO, *P*{*GAL4*-*twi*.*G*}2.2, *P*{*UAS*-2*xEGFP*}*AH2*.2; *ss*¹/+ were again crossed with *w*¹¹¹⁸; *In*(2*LR*) *Gla*, *wg*^{*Gla*-1}/CyO, *P*{*GAL4*-*twi*.*G*}2.2, *P*{*UAS*-2*xEGFP*}*AH2*.2 virgin females. Individual F2 male and virgin female siblings of the genotype *w*¹¹¹⁸; *lys*¹ *rc*¹/CyO, *P*{*GAL4*-*twi*.*G*}2.2, *P*{*UAS*-2*xEGFP*}*AH2*.2 flies were intercrossed to

generate a homozygous *w*¹¹¹⁸; *lys*¹ *rc*¹ strain that lacked *ss*¹. Rescue experiments were conducted by using *da*-*GAL4* to ubiquitously express a previously described *UAS-LKRSDH* transgene (Cakouros *et al.* 2008), which was generously provided by Sharad Kumar (University of South Australia).

The *lys*¹ chromosome also harbored *red cell*¹ (*rc*¹), which is an uncloned recessive mutation that is linked to *lys*¹ on chromosome 2 and located to the right of *dachs*. Homozygous *rc*¹ mutants display ectopic accumulation of a red pigment in adult fat cells. In nearly all of our experiments, both mutant and control animals harbored a single copy of *rc*¹; however, *rc*¹ heterozygotes failed to display the red cell phenotype and *lys*¹ *rc*¹/+ + animals exhibited lysine levels that were similar to both *w*¹¹¹⁸ controls and *LKRSDH*^{*MB01942*}/+ heterozygotes, suggesting that a single copy of *rc*¹ does not influence the lysine phenotype.

Deficiency mapping and complementation tests

The *lys*¹ mutation was mapped by mating males from either *w*¹¹¹⁸ controls or *w*¹¹¹⁸; *lys*¹ *rc*¹ mutants with female flies that harbored molecularly-defined deficiencies (Cook *et al.* 2012). Adult male F1 progeny with straight wings were analyzed for free lysine levels using GC-MS (see below). Complementation tests were conducted using the Minos insertion *Mi*{*ET1*}*LKRSDH*^{*MB01942*} (Bellen *et al.* 2004), which was isolated from BDSC stock #23382. For all mapping experiments and complementation tests, newly eclosed F1 male offspring were aged for 5 days on BDSC food.

GC-MS analysis

Lysine levels were measured using a previously described GC-MS-based method (Tennessen *et al.* 2014). Briefly, 25 adult males were placed in a pretared 2 ml screw cap tube containing 1.4 mm ceramic beads (Catalog No. 15-340-153; Fisher Scientific, Pittsburgh, PA), the mass was determined with an analytical balance, and the tube was immediately dropped into liquid nitrogen. Samples were stored at -80° until processing. Metabolite extraction was achieved using prechilled 90% methanol (HPLC grade) containing 2 µg/ml of succinic-d4 acid as an internal standard, and a two-step derivatization was conducted using 40 mg/ml of methoxyamine hydrochloride in pyridine and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide containing 1% trimethylchlorosilane, respectively. GC-MS analyses for all genetic mapping experiments, complementation tests, and *UAS-LKRSDH* rescue experiments were performed on an Agilent GC6890-5973i MS equipped with a Gerstel MPS autosampler and a 30 m Phenomenex ZB5-5 MSi column. The retention time for lysine in our analysis was 21.6 min, and relative lysine levels were quantified based on the abundance of ions with *m/z* = 317. Values were normalized based on sample mass and the succinic-d4 acid internal standard. The software package Prism 7 version 7.0b (GraphPad Software) was used to statistically analyze targeted lysine measurements and generate scatter plots. The comparison of lysine levels between *w*¹¹¹⁸ and *w*¹¹¹⁸; *lys*¹ *rc*¹ male flies was conducted using a two-tailed, unpaired

Student's *t*-test with Welch's correction. All other genetic experiments were analyzed using one-way ANOVA.

Targeted metabolomic studies were conducted at the University of Utah Metabolomics Core facility, as previously described (Cox *et al.* 2017). This analysis was focused on a set of ~150 metabolites for which fragmentation patterns and retention times were initially established by analyzing chemical standards. In addition, the retention times of all metabolites were calibrated using a series of fatty acid methyl ester standards. Processed data were normalized to both sample mass and a succinic-d4 acid internal standard, and statistically analyzed using MetaboAnalyst 3.0 (metaboanalyst.ca; Xia *et al.* 2015; Xia and Wishart 2016).

PCR amplification of *lys*¹

Tiling fragments of the *lys* locus were amplified and sequenced using a PCR-based strategy (see Supplemental Material, Figure S1 for oligonucleotide sequences). Long-range PCR amplification of exon 2 in the *lys*¹ mutant was conducted using oligonucleotides 5'-AAGTGGTGTTCACAGGTGC-3' and 5'-TGACGACTACCGACCGATATG-3'. Sequencing of the *lys*¹ insertion was conducted using the oligonucleotides 5'-CTGCTTGACCAACTTCTGAC-3' and 5'-GATTACGACTGGGTCCAACCTG-3', which are nested within the PCR product.

Southern blot analysis

Genomic DNA was isolated from *w*¹¹¹⁸ and *lys*¹ adult flies with a Wizard DNA Purification Kit (Promega, Madison, WI) and 5 µg of purified genomic DNA was digested overnight at 37° using *Pst*I (Thermo Fisher Scientific). Southern blot analysis was conducted as previously described (Sullivan *et al.* 2000). A DNA probe corresponding to exon 2 and the surrounding sequence was generated via PCR amplification of *w*¹¹¹⁸ genomic DNA using the oligonucleotides 5'-TGACGACTACCGACCGATATG-3' and 5'-AAGTGACAATCACCAGCAGC-3'.

Quantitative (q)RT-PCR

Total RNA was isolated from 5-day-old adult male flies using Tripure Reagent (Roche). cDNA synthesis was conducted using the Thermo Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase (K1681; Thermo Fisher Scientific). cDNAs and the appropriate oligonucleotides (see below) were added to FastStart Essential DNA Green Master (Roche), and a Roche LightCycler 96 was used to quantify the relative abundance of *LKRSDH*. *rp49* was used as an internal reference. The following primer sets were used to measure the relative abundance of *LKRSDH* mRNA:

rp49 forward: 5'-AAGTGTGCGGCTCGTATTTTCG-3'.
rp49 reverse: 5'-TCATCTTGAAGCAGGTTGGGC-3'.
LKRSDH forward: 5'-ATTGCCAGAGAATCGACGG-3'.
LKRSDH reverse: 5'-TGGCGATAATAGCCGACTGAA-3'.

Data availability

All strains and reagents are available upon request. Table S1 and Table S2 contain the targeted GC-MS metabolomic data,

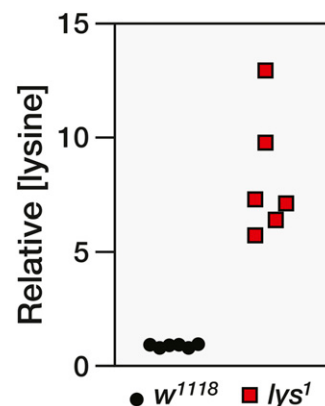


Figure 1 Lysine levels are elevated in *lys*¹ mutants. The relative abundance of lysine was measured in *w*¹¹¹⁸ controls and *w*¹¹¹⁸; *lys*¹ mutants. Each data point represents a single sample that contained 25 adult male flies (*n* = 6 samples per genotype, *P* < 0.001).

which has been normalized to both the sample mass and the succinic-d4 acid internal standard.

Results

Genetic mapping of *lys*¹ using GC-MS

To demonstrate how *Drosophila* intermediate metabolism can be efficiently studied using a combination of metabolomics and forward genetics, we chose to analyze the *lys*¹ mutation, which has no visible phenotype when raised on a standard diet. Since *lys*¹ was last studied in 1961, we used GC-MS to verify that the mutant strain still exhibited abnormally high lysine levels. Our analysis revealed that the levels of lysine are elevated more than fivefold in *lys*¹ mutant males as compared with *w*¹¹¹⁸ controls (Figure 1), thereby confirming that *lys* is an essential regulator of lysine metabolism and demonstrating that we can reliably quantify this phenotype.

E. H. Grell previously mapped the genetic location of *lys*¹ to 2–22.9, which places it to the left of *dachs* on chromosome 2. To further refine the genomic position of *lys*, we crossed *w*¹¹¹⁸ and *lys*¹ male flies with virgin females that harbored a series of overlapping deficiencies (Figure 2A). GC-MS analysis of F1 males revealed that *Df*(2L)*ED508*, *Df*(2L)*ED12527*, and *Df*(2L)*BSC142* failed to complement *lys*¹ (Figure 2), as *trans*-heterozygous offspring exhibited lysine levels that were 10–50-fold higher than those observed in the heterozygous controls (Figure 2B).

Our deficiency mapping results narrowed the location of *lys*¹ to an ~10 kb region containing the genes *LKRSDH* and *Herp* (Figure 3A). Considering that *LKRSDH* encodes an enzyme that catalyzes the first two steps in lysine degradation, we examined the possibility that *lys*¹ is a mutation in this gene. Homozygous *lys*¹ mutants were mated with flies that harbored a Minos insertion in the third exon of *LKRSDH* (*Mi*{*ET1*}*LKRSDH*^{MB01942}) and lysine levels were measured in the resulting F1 progeny. Similar to *lys*¹ homozygous

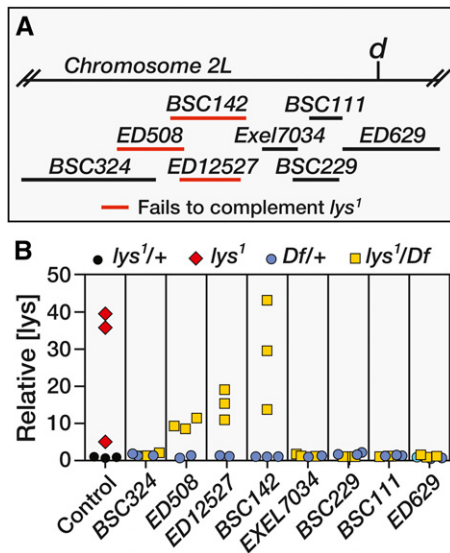


Figure 2 Deficiency mapping of *lys¹*. (A) A schematic diagram illustrating the size and position of the molecularly-defined deficiencies used to genetically map *lys¹*. Homozygous *lys¹* females were crossed with deficiencies (*Df*) that span the genomic region to the left of *dachs* (*d*). Deficiencies highlighted in red failed to complement *lys¹*. (B) GC-MS was used to measure relative lysine levels in 3-day-old adult males. Animals that were heterozygous for either *lys¹* or any of the examined deficiencies exhibited similar lysine levels. In contrast, homozygous *lys¹* mutants and males that were *trans*-heterozygous for *lys¹* and either *Df(2L)ED508*, *Df(2L)ED12527*, or *Df(2L)BSC142* displayed lysine levels that were up to 50-fold higher than controls. Each data point represents a single sample that contained 25 adult male flies. GC-MS, gas chromatography-mass spectrometry.

mutants, adult male flies that were *trans*-heterozygous for *lys¹* and *LKRSDH^{MB01942}* accumulated significantly higher lysine levels than *lys¹* heterozygous controls (Figure 3B). We obtained similar results when either *lys¹* or *LKRSDH^{MB01942}* were placed *in trans* to the deficiency *Df(2L)ED508*, suggesting that both *lys¹* and *LKRSDH^{MB01942}* severely reduce LKRSDH enzyme function. Moreover, ubiquitous expression of a *UAS-LKRSDH* transgene in a *lys¹/LKRSDH^{MB01942}* *trans*-heterozygous background completely rescued the elevated lysine phenotype, demonstrating that the *lys¹* phenotype is due to loss of the LKRSDH enzyme.

To determine the molecular nature of *lys¹*, we used a PCR-based strategy to sequence *LKRSDH* in *lys¹* mutants. During this analysis, we were unable to amplify the second *LKRSDH* exon from *lys¹* genomic DNA using standard PCR techniques, suggesting that a large aberration existed in this region (Figure S1, A and B). We tested this possibility using a Southern blot to examine exon 2 of *LKRSDH* in both *w¹¹¹⁸* control and *lys¹* mutants. A probe corresponding to the *LKRSDH* exon 2 region hybridized with a single ~1.8 kb DNA fragment in the *w¹¹¹⁸* control, but hybridized to two fragments in genomic DNA isolated from the *lys¹* mutant (Figure S1C). Since the mutant bands are collectively larger than the control band, this result suggests that the *lys¹* mutation arises from either a large insertion or an inversion in *LKRSDH*. Indeed,

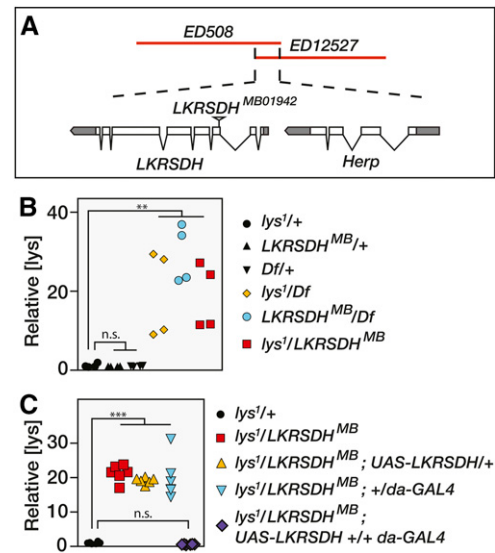


Figure 3 *lys¹* disrupts *LKRSDH*. (A) A schematic diagram of the genomic region deleted by both *Df(2L)ED508* and *Df(2L)ED12527*. The only genes present within this region are *LKRSDH* and *Herp*. The Minos insertion *LKRSDH^{MB01942}* disrupts the third exon of this gene and was used in all subsequent genetic analyses. (B) As compared with heterozygous control strains, lysine levels are elevated between 20- and 40-fold in adult males that harbor a *trans*-heterozygous combination of *lys¹* and either *LKRSDH^{MB}* or *Df(2L)ED508* (*Df*), indicating that *lys¹* disrupts *LKRSDH* function. A similar increase in lysine (*lys*) concentration is observed in males of the genotype *w¹¹¹⁸; LKRSDH^{MB01942}/Df(2L)ED508*. (C) The relative abundance of lysine in adult males of the following genotypes: *w¹¹¹⁸*; *lys¹/+*, *w¹¹¹⁸*; *lys¹/LKRSDH^{MB01942}*, *w¹¹¹⁸*; *lys¹/LKRSDH^{MB01942}; +/da-GAL4*, *w¹¹¹⁸*; *lys¹/LKRSDH^{MB01942}; UAS-LKRSDH/+*, and *w¹¹¹⁸*; *lys¹/LKRSDH^{MB01942}; UAS-LKRSDH +/+ da-GAL4*. (B and C) Each data point represents a single sample that contained 25 adult male flies. n.s., not significant. ** *P* < 0.01 and *** *P* < 0.001.

when we sequenced a long-range PCR product that amplified from the *lys¹* chromosome, we discovered a large insertion in the second exon and a small deletion that removes a portion of the 5'-UTR and the first six amino acids of the enzyme (Figure S1D). Consistent with this finding, qRT-PCR analysis reveals that *lys¹* mutants exhibit an ~80% decrease in *LKRSDH* transcript levels as compared with *w¹¹¹⁸* controls (Figure S1E), indicating that this insertion is a severe loss-of-function allele. Therefore, we will refer to *lys¹* as *LKRSDH¹* for the remainder of this study.

Metabolomic analysis of *LKRSDH* mutants

Although loss-of-function mutations in both the human and *Drosophila* *LKRSDH* homologs fail to produce obvious visible phenotypes, recent observations in the fly suggest that both lysine and LKRSDH are key regulators of physiology and behavior (Cakouros *et al.* 2008; Bjordal *et al.* 2014). In an effort to better understand the role of LKRSDH in *Drosophila* metabolism, we used the same GC-MS method that served as the basis of our genetic analysis to conduct a targeted metabolomic study of *LKRSDH¹/LKRSDH^{MB01942}* mutants and *+/LKRSDH^{MB01942}* controls. Two independent analyses

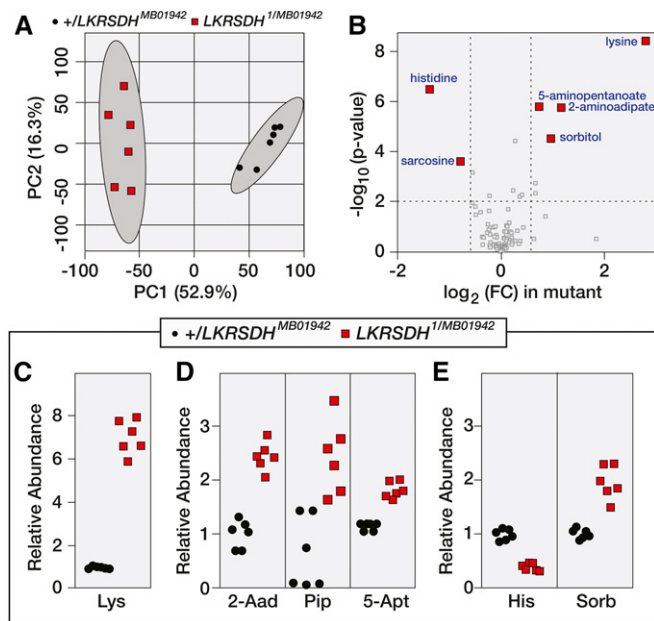


Figure 4 Metabolomic analysis of *LKRSDH* mutants. *w¹¹¹⁸*; *LKRSDH^{MB/+}* and *w¹¹¹⁸*; *LKRSDH^{1/MB01942}* adult males were analyzed using a targeted gas chromatography-mass spectrometry (GC-MS) approach. (A) A comparison of the metabolomic data from control and mutant samples using principle component (PC) analysis. (B) Differences in metabolite abundance between control and mutant samples are represented as a volcano plot. Dashed vertical line represents a fold change (FC) of > 1.5. Dashed horizontal line represents $P < 0.01$. (C–E) The relative abundance of lysine (Lys), 2-aminoadipate (2-Aad), pipecolic acid (Pip), 5-aminopentanoate (5-Apt), histidine (His), and sorbitol (Sorb). Each data point represents a single sample that contained 25 adult male flies. $P < 0.001$ for all panels.

of adult male samples detected between 85 and 100 metabolites (Table S1 and Table S2). A principle component analysis of the resulting data revealed that the mutant strains exhibited a metabolomic profile that was significantly different from controls (Figure 4A and Figure S2A). Moreover, relative lysine concentrations were increased by more than fivefold in *LKRSDH* mutant samples, and lysine was the most significantly altered metabolite in our analysis (Figure 4, B and C and Figure S2B).

In addition to lysine, we observed an unexpected increase in 2-aminoadipate (2Aad; Figure 4, B and D, Figure 5, and Figure S2B), which is a downstream product of lysine catabolism. Since loss of *LKRSDH* activity should inhibit lysine catabolism, we anticipated that 2Aad levels would be decreased in *LKRSDH* mutants. This observed increase in 2Aad suggests that, in the absence of *LKRSDH*, flies produce 2Aad using alternative metabolic pathways. In this regard, our metabolomic data present two possible mechanisms that could bypass the requirement for *LKRSDH* in 2Aad synthesis (Figure 5). First, some animals are thought to be capable of converting lysine into 2Aad via the poorly understood and somewhat controversial pipecolic acid pathway (Figure 5; Broquist 1991; Struys and Jakobs 2010). Furthermore, human patients with familial hyperlysinemia,

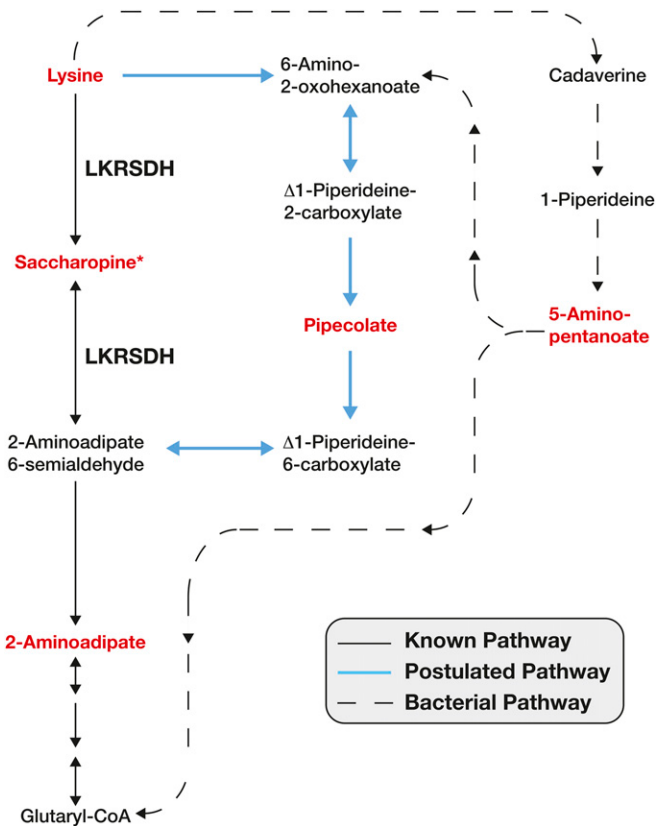


Figure 5 A schematic diagram illustrating the metabolic pathways that catabolize lysine. Metabolites highlighted in red text were measured using a targeted metabolomics approach. Solid black arrows represent the *LKRSDH*-dependent pathway. Blue solid arrows indicate the postulated pipecolic acid pathway. Dashed arrows indicate those metabolic reactions are catalyzed by bacterial enzymes. *, saccharopine was undetectable in both control and mutant samples.

which is caused by mutations in *LKRSDH*, exhibit elevated pipecolic acid levels (Markovitz *et al.* 1984). Since pipecolic acid was not measured during our initial targeted analysis, we reanalyzed our mutants for the presence of this compound and found that pipecolic acid levels were also significantly elevated in *LKRSDH* mutants as compared with controls (Figure 4D). This result indicates that *Drosophila* synthesizes pipecolic acid and suggests that flux through this pathway is increased in *LKRSDH* mutants. Second, *LKRSDH* mutants exhibit elevated 5-aminopentanoate levels (5Apt; Figure 4D, Figure 5, and Figure S2B), which can be produced when lysine is catabolized via a cadaverine intermediate (Fothergill and Guest 1977). While there is some evidence that animals can directly convert lysine to cadaverine by an unknown mechanism, the cadaverine pathway is most commonly associated with bacterial and plant metabolism (Miller-Fleming *et al.* 2015). Therefore, these results hint at the possibility that either the fly microbial community is contributing to lysine catabolism in *LKRSDH* mutants or that *Drosophila* produces this compound by an uncharacterized metabolic mechanism.

In addition to the metabolic changes associated with lysine catabolism, our metabolomic analysis of *LKRSDH* mutants also revealed reproducible changes in histidine, sorbitol, and sarcosine. The decrease in histidine concentration is particularly notable, as after lysine, histidine is the most altered metabolite in *LKRSDH* mutants. However, the metabolic relationships between lysine and these three compounds remain unknown and represent novel metabolic links that could only be discovered using a metabolomics approach.

Discussion

Here, we demonstrate how a combination of forward genetics and metabolomics can be used to rapidly identify and characterize defects in *Drosophila* intermediate metabolism. In our study, we genetically mapped and characterized defects associated with *lys*¹, a mutant with no obvious morphological defects. The fact that we could conduct a metabolomic analysis of our mutants with the same method used for mapping *LKRSDH*¹ also allowed us to rapidly identify metabolic phenotypes caused by this mutation. Our efforts revealed that *LKRSDH* mutants appear to compensate for loss of LKRSDH activity by utilizing the poorly understood pipecolic acid pathway, which is also active in human patients with familial hyperlysinemia (Markovitz *et al.* 1984). Furthermore, the elevated levels of 5Apt in *LKRSDH* mutants indicate that either flies are capable of synthesizing 5Apt via an unknown metabolic pathway or that lysine levels are controlled, in part, by metabolic cross talk between somatic tissues and the fly microbiome. This latter possibility is supported by a recent study that demonstrated that the fly bacterial community is intimately associated with host amino acid metabolism (Leitao-Goncalves *et al.* 2017). Overall, our findings demonstrate that both flies and humans use similar metabolic mechanisms to catabolize lysine, thereby establishing *Drosophila* as a model for both investigating mechanisms of pipecolic acid synthesis and studying potential cross talk between this poorly understood pathway, bacterial metabolism, and LKRSDH.

While our analysis provides an initial metabolic characterization of *LKRSDH*, lysine was previously shown to regulate larval feeding behavior, and LKRSDH is also known to moonlight as a regulator of ecdysone signaling (Cakouros *et al.* 2008; Bjordal *et al.* 2014). Yet, despite these roles for lysine metabolism in development and physiology, *LKRSDH* mutations were never isolated in genetic screens for visible phenotypes. The fact that *LKRSDH* was overlooked by genetic studies is consistent with the fact that *Drosophila* development can tolerate severe metabolic disruptions. For example, *Mitochondrial Pyruvate Carrier 1* mutants are viable despite being unable to transport pyruvate into their mitochondria (Bricker *et al.* 2012), the oxidative branch of the pentose phosphate pathway is dispensable under standard culture conditions (Hughes and Lucchesi 1977), and *Malate Dehydrogenase* mutants, which lack a functional citric acid cycle,

grow at a normal rate during larval development (Wang *et al.* 2010). If major disruptions of intermediate metabolism fail to elicit easily recognizable phenotypes, then subtle metabolic regulators will be nearly impossible to identify and study based on morphological or behavioral defects. In contrast, a metabolomic approach that uses metabolites as a primary phenotypic readout would quickly identify any of the mutants described above and provide a rapid and reliable means to characterize their metabolic functions.

The power of using metabolomics in forward genetics studies was recently demonstrated by an analysis of the *Saccharomyces cerevisiae* gene knockout collection (Mulleder *et al.* 2016). This study revealed that one-third of coding genes influence the concentration of at least one amino acid, and of the ~1000 unstudied genes affecting amino acid metabolism, 440 have human homologs, thereby revealing our surprising lack of knowledge regarding the regulation of eukaryotic intermediate metabolism. Previous metabolomic studies in flies suggest that the regulation of animal metabolism is similarly complex (Cox *et al.* 2017), indicating that a large-scale metabolomic analysis of the available *Drosophila* mutant and RNA interference collections has the potential to dramatically expand our understanding of eukaryotic metabolism.

Finally, our study highlights the work of E. H. Grell, who discovered the *lys*¹ mutation as the result of a series of serendipitous observations (Grell 1958). At the time, the *lys*¹ phenotype could be scored only by chromatography or based on its ability to enhance the phenotype of *red cell* mutations. The fact that Grell identified and mapped *lys*¹ is a testament to the tenacity of *Drosophila* geneticists. In addition, Grell conducted a series of unpublished experiments suggesting that *lys*¹ mutants are able to catabolize both pipecolic acid and 2Aad (Grell 1958). While unknown at the time, this observation pinpointed LKRSDH as the origin of the *lys*¹ phenotype (see Figure 5). Fifty years later, our analysis of *lys*¹ both provides closure to Grell's observations and demonstrates how emerging metabolomic technologies can be used in forward genetic studies.

Acknowledgments

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Literature Cited

- Bellen, H. J., R. W. Levis, G. Liao, Y. He, J. W. Carlson *et al.*, 2004 The BDGP gene disruption project: single transposon insertions associated with 40% of *Drosophila* genes. *Genetics* 167: 761–781.
- Bjoridal, M., N. Arquier, J. Kniazeff, J. P. Pin, and P. Leopold, 2014 Sensing of amino acids in a dopaminergic circuitry promotes rejection of an incomplete diet in *Drosophila*. *Cell* 156: 510–521.
- Bricker, D. K., E. B. Taylor, J. C. Schell, T. Orsak, A. Boutron *et al.*, 2012 A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, *Drosophila*, and humans. *Science* 337: 96–100.
- Broquist, H. P., 1991 Lysine-pipecolic acid metabolic relationships in microbes and mammals. *Annu. Rev. Nutr.* 11: 435–448.
- Cakouras, D., K. Mills, D. Denton, A. Paterson, T. Daish *et al.*, 2008 dLKR/SDH regulates hormone-mediated histone arginine methylation and transcription of cell death genes. *J. Cell Biol.* 182: 481–495.
- Cook, R. K., S. J. Christensen, J. A. Deal, R. A. Coburn, M. E. Deal *et al.*, 2012 The generation of chromosomal deletions to provide extensive coverage and subdivision of the *Drosophila melanogaster* genome. *Genome Biol.* 13: R21.
- Cox, J. E., C. S. Thummel, and J. M. Tennessen, 2017 Metabolomic studies in *Drosophila*. *Genetics* 206: 1169–1185.
- Doane, W. W., 1960 Developmental physiology of the mutant *female sterile(2)adipose* of *Drosophila melanogaster*. I. Adult morphology, longevity, egg production, and egg lethality. *J. Exp. Zool.* 145: 1–21.
- Fothergill, J. C., and J. R. Guest, 1977 Catabolism of L-lysine by *Pseudomonas aeruginosa*. *J. Gen. Microbiol.* 99: 139–155.
- Grell, E. H., 1958 Genetics and biochemistry of “red cell”, pp. 58 in *Drosophila melanogaster*. California Institute of Technology, Pasadena, CA.
- Grell, E. H., 1961 The genetics and biochemistry of red fat cells in *Drosophila melanogaster*. *Genetics* 46: 925–933.
- Hader, T., S. Muller, M. Aguilera, K. G. Eulenberg, A. Steuernagel *et al.*, 2003 Control of triglyceride storage by a WD40/TPR-domain protein. *EMBO Rep.* 4: 511–516.
- Hughes, M. B., and J. C. Lucchesi, 1977 Genetic rescue of a lethal “null” activity allele of 6-phosphogluconate dehydrogenase in *Drosophila melanogaster*. *Science* 196: 1114–1115.
- Leitao-Goncalves, R., Z. Carvalho-Santos, A. P. Francisco, G. T. Fioreze, M. Anjos *et al.*, 2017 Commensal bacteria and essential amino acids control food choice behavior and reproduction. *PLoS Biol.* 15: e2000862.
- Lindsley, D., and G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, Inc., San Diego, CA.
- Markovitz, P. J., D. T. Chuang, and R. P. Cox, 1984 Familial hyperlysinemias. Purification and characterization of the bifunctional amino adipic semialdehyde synthase with lysine-ketoglutarate reductase and saccharopine dehydrogenase activities. *J. Biol. Chem.* 259: 11643–11646.
- Miller-Fleming, L., V. Olin-Sandoval, K. Campbell, and M. Ralser, 2015 Remaining mysteries of molecular biology: the role of polyamines in the cell. *J. Mol. Biol.* 427: 3389–3406.
- Mulleder, M., E. Calvani, M. T. Alam, R. K. Wang, F. Eckerstorfer *et al.*, 2016 Functional metabolomics describes the yeast biosynthetic regulome. *Cell* 167: 553–565.e12.
- Pospisilik, J. A., D. Schramek, H. Schnidar, S. J. Cronin, N. T. Nehme *et al.*, 2010 *Drosophila* genome-wide obesity screen reveals hedgehog as a determinant of brown vs. white adipose cell fate. *Cell* 140: 148–160.
- Reis, T., M. R. Van Gilst, and I. K. Hariharan, 2010 A buoyancy-based screen of *Drosophila* larvae for fat-storage mutants reveals a role for Sir2 in coupling fat storage to nutrient availability. *PLoS Genet.* 6: e1001206.
- Song, W., D. Cheng, S. Hong, B. Sappe, Y. Hu *et al.*, 2017 Midgut-derived activin regulates glucagon-like action in the fat body and glycemic control. *Cell Metab.* 25: 386–399.
- Struys, E. A., and C. Jakobs, 2010 Metabolism of lysine in alpha-amino adipic semialdehyde dehydrogenase-deficient fibroblasts: evidence for an alternative pathway of pipecolic acid formation. *FEBS Lett.* 584: 181–186.
- Suh, J. M., D. Zeve, R. McKay, J. Seo, Z. Salo *et al.*, 2007 *Adipose* is a conserved dosage-sensitive antiobesity gene. *Cell Metab.* 6: 195–207.
- Sullivan, W., M. Ashburner, and R. Hawley, 2000 Laboratory Culture of *Drosophila*. pp. 589–590 in *Drosophila Protocols*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Teague, B. D., A. G. Clark, and W. W. Doane, 1986 Developmental analysis of lipids from wild-type and *adipose60* mutants of *Drosophila melanogaster*. *J. Exp. Zool.* 240: 95–104.
- Tennessen, J. M., W. E. Barry, J. Cox, and C. S. Thummel, 2014 Methods for studying metabolism in *Drosophila*. *Methods* 68: 105–115.
- Ugrankar, R., E. Berglund, F. Akdemir, C. Tran, M. S. Kim *et al.*, 2015 *Drosophila* glucone screening identifies Ck1alpha as a regulator of mammalian glucose metabolism. *Nat. Commun.* 6: 7102.
- Wang, L., G. Lam, and C. S. Thummel, 2010 *Med24* and *Mdh2* are required for *Drosophila* larval salivary gland cell death. *Dev. Dyn.* 239: 954–964.
- Xia, J., and D. S. Wishart, 2016 Using MetaboAnalyst 3.0 for comprehensive metabolomics data analysis. *Curr. Protoc. Bioinformatics* 55: 14.10.1–14.10.91.
- Xia, J., I. V. Sinelnikov, B. Han, and D. S. Wishart, 2015 MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic Acids Res.* 43: W251–W257.

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