FEBS Open Bio 2 (2012) 217–221

FEBS openbio

journal homepage: www.elsevier.com/locate/febsopenbio

Metabolomic profiling reveals that *Drosophila melanogaster* larvae with the *y* mutation have altered lysine metabolism

Mohammed Al Bratty [a], Venkateswara R. Chintapalli [b], Julian A.T. Dow [b], Tong Zhang [a], David G. Watson [a,*]

[a] Strathclyde Institute of Pharmacy and Biomedical Sciences, 161, Cathedral Street, Glasgow G4 0RE, UK
[b] Institute of Cell, Molecular and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK

ARTICLE INFO

Article history:
Received 21 June 2012
Revised 19 July 2012
Accepted 20 July 2012

Keywords:
Fourier transform mass spectrometry
Histones
Metabolomics
Saccharopine
Yellow mutation

ABSTRACT

Yellow (*y*) encodes a protein which is closely similar to major royal jelly proteins produced by bees. However, the function of *y* remains largely unknown. Metabolomic profiling was carried out on third instar Oregon R (OR) and *yellow* (*y*) *Drosophila melanogaster* larvae. Phenylalanine, tyrosine and DOPA were all elevated in *y* as might be expected since the mutation blocks melanin biosynthesis. The most consistent effects were related to lysine metabolism, with the lysine metabolite saccharopine being much higher in *y*. In addition, lysine acetate was elevated, and the levels of methyl lysines were lower, in *y* than in OR.

1. Introduction

Yellow (*y*) was one of the first *Drosophila* mutants discovered [1] and encodes a multifunctional protein with close similarity to the major royal jelly protein of honeybees. According to the *Drosophila* gene expression database, FlyAtlas.org [2], *y* is specifically expressed only in larval salivary glands, suggesting a secretory or digestive function. Royal jelly is secreted from analogous glands in the heads of honeybee workers, suggesting a conserved role for *y*. It has been proposed that in the honeybee *y* proteins may regulate development epigenetically by promoting DNA methylation [3]. *Y* is also extensively expressed in the pupal cuticle, where it plays a major role in melanisation [4]; and expression in the CNS is required for normal male reproductive behaviour [5–7]. So *y* appears to play multiple roles, although its exact biochemical modes of action are still unknown.

It has been proposed that Yellow is a structural protein and that it is involved in forming cross-links with the dopamine derivative indole-5,6-quinone during melanisation [5]. Another theory is that it acts downstream of dihydroxyphenylalanine (DOPA) in the formation of melanin; there is some sequence homology between the *y* protein and dopachrome conversion enzyme (DCE) [8].

In previous papers we have looked at the effects of a known genetic lesion *rosy* on the global metabolite profile in *Drosophila* and found effects on metabolites remote from those directly affected by the lesion [9] and also attempted to explain an unknown genetic lesion [10]. We report here the application of metabolomics in order to elucidate the effects of the *y* gene.

2. Materials and methods

2.1. Chemicals and solvents

HPLC grade acetonitrile, formic and water were obtained from Fisher Scientific, UK. All other chemicals were from Sigma–Aldrich, UK.

2.2. Fly larvae

Laboratory grown wild-type Oregon R strain of *Drosophila melanogaster* was used as the control for the *y1* amorphic mutant (Bloomington Stock Centre, Indiana, #169). Larvae were raised on standard medium on a 12:12 h L:D cycle, at 23 °C, and at 55% relative humidity. For metabolomic analysis, feeding third-instar larvae (before they stop feeding and climb away from their food) were selected. Ice cold methanol/chloroform/water (3:1:1, 250 μl) was used in the sample quenching and extraction method. Ten larvae from both genders were collected from the food for each biological replicate. First the larvae were rinsed in phosphate buf-

Abbreviations: y, yellow; OR, Oregon R; dLKR, *Drosophila* lysine ketoglutarate reductase; SDH, saccharopine dehydrogenase; DCE, dopachrome conversion enzyme; DHI, 5,6-dihydroxyindole; DOPA, dihydroxyphenylalanine

* Corresponding author. Tel.: +44 1415482651.
E-mail address: d.g.watson@strath.ac.uk (D.G. Watson).

2211-5463/$36.00
http://dx.doi.org/10.1016/j.fob.2012.07.007

fer saline (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4 and 1.47 mM KH_2PO_4; adjusted to a final pH of 7.4), blotted dry, then homogenised in the solvent mixture, followed by 10 s of sonication. The homogenate was then centrifuged for 10 min at 4 °C. The supernatant was collected and stored at −80 °C until analysis. A total of four biological replicates containing 10 mixed male and female larvae was collected for each strain at each time point.

2.3. DNA/RNA hydrolysis

DNA/RNA was extracted from 100 larvae for each biological replicate using the phenol–chloroform extraction method [11]. A total of four biological replicates were prepared for both Oregon R and the *y* mutant. Briefly, the DNA extraction procedure was as follows: whole larvae were ground in a 1.5 ml microcentrifuge tube using a hand pestle and subsequently sonicated briefly using a Microson Ultrasonic cell disruptor (Misonix Inc, USA) in 400 μl buffer A (100 mM Tris–HCl (pH 7.5), 100 mM EDTA, 100 mM NaCl, 0.5% SDS). After incubation at 65 °C for 30 min, 800 μl of LiCl/KAc mixture (1 part 5 M KAc:2.5 parts 6 M LiCl) was added. The samples were spun for 15 min at room temperature (RT) at maximum speed in a table-top microcentrifuge. Floating curd was removed using a pipette tip and 1 ml of supernatant was taken into a new tube. Then, 600 μl of isopropanol was added and the sample was mixed using a pipette. Another round of centrifugation was performed at RT for 15 min. The supernatant was discarded and the pellet was washed with 70% ethanol and dried for 15 min at RT. Dried pellet was re-suspended in 100 μl of nuclease-free water. DNA/RNA was quantified using a NanoVue plus spectrophotometer (GE Life Sciences, UK).

For DNA/RNA hydrolysis, a method was adapted from Kriaucionis et al. [12]. Briefly, 100 μg of DNA/RNA were incubated in the hydrolysis solution (100 mM NaCl, 20 mM $MgCl_2$, 20 mM Tris pH 7.9, 1 U/μl Benzonase™ endonuclease, 600 mU/ml phosphodiesterase I, 80 U/ml alkaline phosphatase, 36 μg/ml EHNA-hydrochloride, 2.7 mM desferoxamine). Hydrolysed DNA/RNA was filtered using an Amicon microcon 10 kDa cut-off centrifugal filter unit (Millipore UK Ltd.). The hydrolysate was used for LC–MS analysis after diluting (1:4) with acetonitrile.

2.4. Nuclear protein isolation

For histone analysis, the nuclear protein fraction was extracted using NE-PER nuclear extraction reagent kit (Thermo Scientific, UK). Briefly, 50 larvae were washed in phosphate buffer saline (PBS) 3 times. Using a pipette, all the residual PBS was removed and the larvae were homogenized at 4 °C in ice-cold CER I buffer (including 1:100 protease inhibitor cocktail from Sigma–Aldrich, UK). The rest of the extraction was performed according to the manufacturer protocol. Finally the nuclear fraction was collected and protein quantity was measured using a Bradford calorimetric protein assay kit (BIO-RAD, UK). A total of 80 μg of nuclear protein was used for the hydrolysis as follows. The protein was mixed with 160 μl of 6M HCl in a vial. The vial was sealed and heated in an oven at 145 °C for 4 h. The mixture was cooled and 160 μl of 3 M sodium bicarbonate was added. Then 0.4 ml of acetonitrile was added to a protein crash plate (Biotage Ltd, Sweden) followed by 0.2 ml of the acid hydrolysate. Salts were allowed to precipitate for 10 min and then the sample was filtered. The filtrate was used for LC–MS analysis.

2.5. LC–MS method

LC–MS data were acquired using an LTQ Orbitrap instrument (Thermo Scientific, UK) set at 30,000 resolution. Sample analysis was carried out under both positive and negative ion modes. The mass scanning range was *m*/*z* 50–1200, the capillary temperature was 200 °C and the sheath and auxiliary gas flow rates were 30 and 10 arbitrary units respectively. The LC–MS system (controlled by Xcalibur version 2.0, Thermo) was run in binary gradient mode. Solvent A was 0.1% v/v formic acid in HPLC grade water and solvent B was 0.1% v/v formic acid in acetonitrile. Analysis was carried out on a ZICHILIC column (150 × 4.6 mm, 5 μm particle size, HiChrom Ltd., UK) fitted with a guard column. A flow rate of 300 μl/min was used and the injection volume was 10 μl, the gradient used was as follows: 90% B at (0 min) – 50% B at (16 min) – 20% B at (18 min) – 20% B at (28 min) – 90% B at (36 min). Samples were kept in a vial tray which was set at a constant temperature of 3 °C. The injection volume was 25 μl.

The instrument was calibrated according to the manufacturer's instructions just before commencing the experiment, and was internally calibrated by lock masses (positive ion mode *m*/*z* 83.06037 and *m*/*z* 195.08625, due to acetonitrile dimer and caffeine respectively; and negative ion mode 91.00368 due to formic acid dimer). Runs were carried out in negative ion mode using the conditions above after tuning in the negative ion mode and the assigning appropriate lock mass. Runs were also carried out on an Orbitrap Exactive instrument fitted with a HCD cell using the same mass spectrometry parameters and chromatographic conditions with a HCD cell energy of 20 eV.

2.6. Data processing

Data files were processed using Sieve 1.3 (Thermo, UK). The parameters used in Sieve were: time range 4–30 min, mass range 75–700 amu, frame width 0.02 amu and Rt width 2.5 min. The output from Sieve was transferred into Sieve Extractor, an Excel spreadsheet and an in-house macro written in Visual Basic, and used to search against a mass list of 35,000 compounds taken from the KEGG, Metlin, Human Metabolome and Lipid Maps databases. A 3 ppm window was set for assignment of elemental composition and often there was only one chemically sensible elemental composition within this window. Matching against the database confirmed this where only one hit was returned or if more than one hit was returned then the additional hits were isomers. Sieve software measures *P*-values using a two-tailed Student's *t*-test. We have found good agreement for selected compounds between manually calculated values and the values returned by the software. Profiling was based on for samples of larvae taken at three time points during two months ($n = 4$ at each sampling point). Repeat sampling gives more confidence in the stability of differences over time. It is not possible to combine results from separate sampling points because of variations in instrument sensitivity and chromatography; relatively small variations in chromatographic retention time make peak alignment difficult.

3. Results and discussion

Table 1 summarises some putatively identified unusual metabolites present in the larvae and also metabolites where there is a marked variation between *y* and OR. Novel metabolites were individually curated, requiring a mass accuracy within 1.5 ppm of the proposed structure, and with no sensible elemental composition within 3 ppm of the assigned elemental composition based on the elements C, N, H, O, P and S. All the metabolites listed in Table 1 were also checked manually to confirm that they were not isotope peaks, adducts, fragments or dimers of more intense ions. Table S1 (supplementary) shows the MS^2 data for some the more unusual metabolites which helps to confirm their structures.

y is a visible mutant of melanisation, and appears to lack homology to known enzymes in the tyrosinase/dopa pathways, but in-

Table 1
List of unusual metabolites and metabolites displaying significant differences (n = 4 at each sampling point) between y and OR larvae. Four extracts from 10 *y* and 10 OR mixed male and female larvae were prepared on 3 occasions over a two month time period. "Ratio" is of *y*: OR peak area.

Compounds	MH^+/M^-	Rt	Replicate 1		Replicate 2		Replicate 3	
		min	Ratio	*P* value	Ratio	*P* value	Ratio	*P* value
Melanin biosynthesis								
Dihydroxyindole	150.0545	8.0	3.30	1.80E−02	4.15	1.90E−02	2.27	5.50E−02
Phenylalanine	166.0864	12.4	2.21	7.10E−06	2.18	3.90E−03	1.99	3.10E−04
Tyrosine	182.081	14.9	1.73	5.90E−03	2.12	9.30E−02	3.32	1.70E−04
Dopamine acetate	196.097	7.5	0.52	1.70E−03	2.30	1.80E−01	0.523	4.00E−01
DOPA	198.0761	17.3	2.67	3.00E−02	3.29	1.30E−03	3.229	5.20E−03
Glycyl-dopa	255.0978	17.1	1.34	2.10E−01	1.96	1.40E−01	1.98	5.80E−02
Dihydroxy indole GSH	455.1231	17.2	0.90	8.20E−01	ND		0.64	3.30E−01
Lysine metabolism								
Lysine	147.1129	23.4	2.23	1.20E−02	2.07	3.70E−02	1.62	3.90E−02
Methyllysine	161.1287	23.0	0.62	7.70E−04	0.72	4.30E−02	0.83	2.70E−01
2-Aminoadipate	162.0763	16.1	2.00	2.20E−02	2.28	2.90E−02	1.38	1.30E−01
Carnitine	162.1127	15.4	0.60	2.30E−08	0.60	6.90E−04	0.81	1.50E−02
Dimethyl lysine	175.1442	23.1	0.41	3.00E−05	0.39	1.80E−04	0.56	2.40E−02
Lysine acetate	189.1236	16.4	6.19	4.70E−03	4.50	5.00E−03	3.16	4.70E−05
Trimethyl lysine	189.1599	23.8	0.36	3.30E−03	0.45	2.00E−03	0.61	1.30E−01
Diaminopimelate	191.1028	16.6	0.66	1.30E−02	0.51	7.90E−03	0.77	2.80E−03
Aspartyl lysine	262.1397	21.5	0.01	5.80E−05	0.05	1.20E−01	0.064	7.50E−03
Saccharopine	277.1395	20.8	8.20	1.80E−02	9.50	5.80E−03	5.093	3.70E−02
Fructosyl lysine	309.1658	23.8	0.47	3.70E−03	0.78	3.30E−02	0.87	5.00E−01
Chitin biosynthesis								
N-acetylglycosamine	222.0975	13.5	0.10	3.20E−02	0.50	5.80E−01	0.198	2.00E−01
N-acetylglycosamine isomer	222.0975	15.0	0.06	3.00E−02	0.54	6.7 E−01	0.045	2.10E−01
Glycosamine phosphate isomer	260.0532	19.9	1.55	4.20E−03	0.95	6.30E−01	0.374	2.30E−01
Glycosamine phosphate isomer	260.0532	22.2	0.14	6.50E−04	ND	ND	0.024	4.70E−03
Neuraminic acid	268.1027	20.0	1.71	5.70E−04	3.44	4.40E−04	2.003	2.00E−03
Glycosamine acetate phosphate	302.0638	19.8	0.35	1.20E−02	0.66	4.60E−02	0.58	2.30E−03
Cytidine monophosphate	324.0594	20.4	2.40	3.20E−02	7.77	9.80E−03	3.51	4.20E−02
UDP Glc NAc	608.0886	25.8	1.16	1.70E−01	2.01	4.50E−02	1.812	6.50E−03
Tryptophan metabolism								
Kynurenic acid	190.05	8.9	2.52	4.80E−03	2.29	4.10E−03	3.289	2.00E−04
Xanthurenic acid	206.0449	10.6	2.31	8.90E−03	2.68	5.90E−02	7.904	2.90E−03
Hydroxytryptophan	221.0923	12.8	0.35	4.50E−02	0.12	4.60E−03	0.26	6.30E−02
Hydroxykynurenine	225.087	14.2	0.40	3.70E−06	0.35	8.70E−04	0.43	1.10E−02
GSH oxidative stress								
Methionine S-oxide	166.0534	18.3	6.29	3.40E−03	3.78	1.80E−02	3.26	2.00E−04
Cystathione	223.0749	21.8	2.13	5.00E−02	2.71	2.90E−02	1.81	1.80E−01
GSH	308.0912	16.3	0.05	9.50E−06	0.00	3.60E−01	0.01	3.30E−01
GSH-cysteine	427.0954	21.0	0.43	1.10E−02	0.41	2.60E−01	0.34	2.20E−01
GSSG	613.1594	20.5	0.52	7.10E−02	0.48	2.10E−01	0.26	1.70E−01
Methylation								
Methionine	150.0584	13.9	2.52	2.00E−03	1.47	9.60E−02	1.70	2.90E−02
S-adenosylmethionine	399.1448	24.6	2.25	5.20E−03	2.13	1.50E−02	2.263	1.70E−03
Purine metabolism								
Guanine	152.0544	13.9	2.41	4.10E−03	1.39	1.60E−01	1.50	6.20E−02
Sepiapterin	238.0936	10.3	0.03	2.90E−03	0.06	1.90E−02	0.246	1.90E−01
Biopterin	238.0938	13.2	0.63	1.20E−04	0.56	1.70E−02	0.311	2.20E−01
Dihydrobiopterin	240.1093	13.9	0.25	6.50E−04	0.18	6.90E−04	0.263	2.00E−02
Methyladenosine	282.1185	19.4	2.70	2.80E−03	10.70	3.80E−03	1.02	8.80E−01
Methylguanosine	298.1146	11.1	0.43	3.90E−04	0.25	1.60E−03	0.552	5.20E−02
Misc. metabolites								
Threonine	120.0657	17.4	1.96	7.50E−03	2.43	1.10E−02	1.95	8.20E−03
Asparagine	133.0609	18.5	0.49	1.90E−02	0.45	8.30E−03	0.34	2.00E−03
Glutamine	147.0765	18.2	0.60	1.70E−02	0.55	7.60E−03	0.71	1.50E−04
Histidine	156.0769	22.6	0.63	1.00E−04	0.65	1.40E−02	0.61	4.20E−02
Choline phosphate	184.0734	22.4	0.32	2.00E−03	0.30	9.60E−06	0.41	9.50E−06
Dimethylarginine	203.1391	22.5	0.22	2.30E−02	ND	-	0.61	3.10 E−02
Cytidine	244.093	17.8	1.66	1.80E−02	2.59	6.70E−03	0.485	1.40E−02
Uridine	245.077	10.8	1.17	2.90E−01	2.17	6.80E−02	0.674	2.60E−03
Arginine phosphate	255.0853	22.8	0.56	7.00E−04	0.35	1.40E−04	0.326	3.20E−03
Tyrosine phosphate	262.0475	20.7	1.06	3.50E−01	1.48	1.60−E02	0.264	3.60E−02
Arginosuccinate	291.1301	22.0	0.38	1.20E−03	0.45	1.60E−02	0.115	7.30E−03
Riboflavin	377.1454	9.5	3.23	1.10E−03	2.65	2.10E−02	2.842	1.40E−04

stead supports the activity of dopachrome-converting enzymes Yellow-f and Yellow-f2 [8]. As might be expected there is thus a marked effect of the absence of *y* protein on metabolites in the pathway of eumelanin biosynthesis (Fig. 1) with phenylalanine, tyrosine, DOPA and dihydroxyindole (DHI) consistently accumulating in all three batches of *y* larvae analysed. A widespread theory of the action of *y* is that it governs dopachrome conversion. However, the presence of DHI in *y*, which is an immediate precursor of eumelanin, indicates that it is not simply lack of a precursor which blocks eumelanin biosynthesis. The only enzymatic step between DHI and eumelanin is catalysed by tyrosinase which is used twice in the pathway leading to DHI. A clue to the fate of DHI not used in

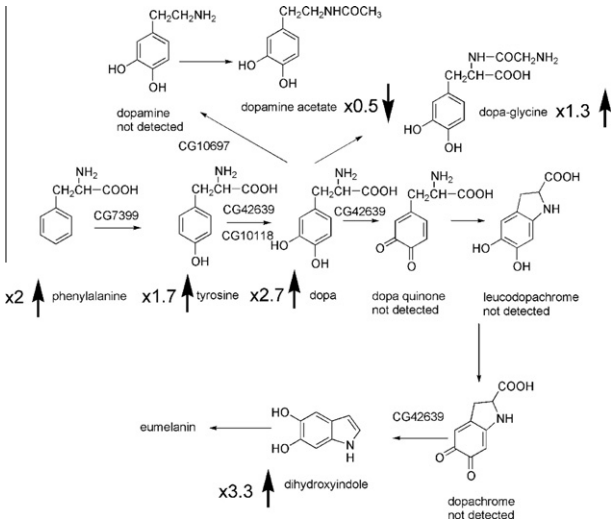

Fig. 1. Impact of *y* mutation on eumelanin biosynthesis with known *Drosophila* enzymes indicated, and fold changes for metabolites for *y* versus OR (from Table 1) shown in arrows.

melanin biosynthesis is given by the presence of relatively abundant peaks which correspond to the glutathione adducts of DHI (Fig. 2); the multiple peaks are due to reaction at more than one position in the ring system. The levels of DHI-GSH do not differ between *y* and OR. Apart from the eumelanin biosynthesis pathway there are number of other metabolic pathways that are strikingly affected in *y*.

The most metabolites within a single pathway that are affected by the mutation are related to lysine metabolism (Fig. 3). A major metabolite of lysine in the larvae is fructosyl lysine which has only been reported as a non-enzymatic glycation product deriving from the reaction between lysine residues within proteins and glucose [13]. Lysine is consistently elevated in *y* and the lysine metabolite saccharopine is elevated between 5 and 10 times compared to OR. The elevated levels of saccharopine are interesting in view of the recent finding that the ketoglutarate reductase (dLKR)/saccharopine dehydrogenase (SDH), the enzyme responsible for saccharopine formation, has been found to suppress ecdysone-mediated cell death [14]. dLKR/SDH suppresses the activity of ecdysone by binding to histones H3 and H4 thus inhibiting arginine methylation within the histone tails. In *y*, lysine acetate levels are elevated >x3 and, when compared with OR, and methyl-, dimethyl- and trimethyllysine are two to three times lower than in OR. Both acetyllysine and methylated lysines are derived from turnover of histones and provide two strategies controlling chromatin remodelling and thus gene transcription. Acetylation of lysine removes

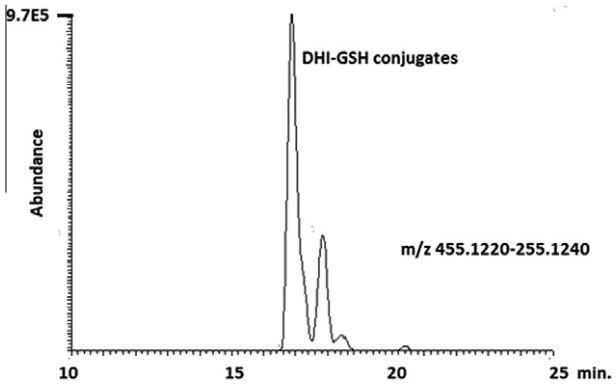

Fig. 2. Extracted ion trace showing the putatively identified GSH conjugates of DHI.

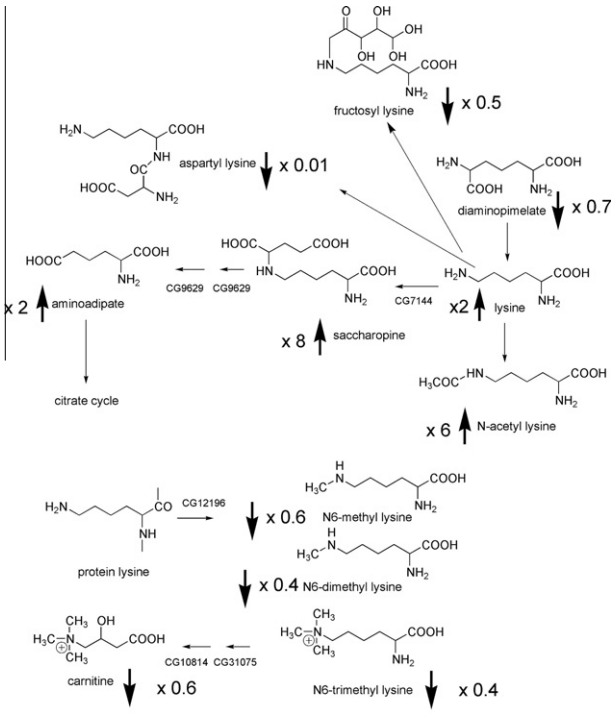

Fig. 3. Impact of *y* mutation on lysine catabolism with known *Drosophila* enzymes indicated, and fold changes for metabolites for *y* versus OR (from Table 1) shown in arrows. Note that there are no enzymes present in drosophila capable of synthesizing lysine; as in other higher animals, lysine is an essential amino acid.

the electrostatic interaction between the lysine residues in histone and the phosphate backbone of DNA allowing access of transcriptional enzymes [15]. *y* larvae also contain elevated levels of aminoadipate which is the next step in the lysine degradation pathway. Tryptophan metabolism via the kynurenine pathway is also affected with hydroxykynurenine levels being lower in *y* and levels of the downstream metabolites in this pathway kynurenic acid and xanthurenic acid being elevated. Both kynurenic and xanthurenic acids are produced by the enzyme L-kynurenine:2-oxoglutarate aminotransferase (KOA). The gene coding for this enzyme is orthologous with 2-aminoadipate transaminase which is responsible for the formation of oxoadipate from aminoadipate in the lysine degradation pathway. Thus increased levels of kynurenic acid and xanthurenic acid may be linked to the increase in lysine degradation products.

Several metabolic changes observed in *y* larvae were consistent with variation in histone modification. Histone proteins are steadily turned over [16] and might be expected to contribute modified methyllysines and dimethylarginine to the metabolome. In addition, methionine and *S*-adenosyl methionine accumulate in *y* as if they were being underutilised. This suggests some differences in the regulation of methylation between OR and *y*. In order to determine whether or not *y* is directly involved in controlling the level of methylation of histone proteins, histones were isolated from OR and *y* larvae and subjected to a simple acid hydrolysis. Although the isolated proteins were rich in methylated lysine residues, particularly trimethyl lysine, there were no significant differences in the degree of methylation of histones from *y* and OR. In addition to methylated lysines there was an abundance of dimethylarginine present in the hydrolysate from the histones but there was no significant difference between dimethyl arginine in hydrolysates from *y* and OR histones. Table S2 (supplementary) summarises the data obtained for methyllysines and arginine in OR and *y* normalised to the mean areas for the peaks for lysine or arginine in the hydrolysate.

Since it has been proposed that royal jelly proteins may be involved in epigenetic modification of DNA via methylation [17] and it was decided to examine DNA isolated from OR and *y*. DNA/RNA was isolated from OR and *y* larvae and enzymatically digested to yield free DNA/RNA bases and then analysed by LCMS. All of the bases detected were derived from RNA and contained ribose rather than deoxyribose and no modified DNA bases were detected. It was possible to see methylated bases to a level of *ca* 0.1% of the non-methylated bases (Fig. S1 supplementary), there was no significant difference in the levels of methylated RNA bases between OR and *y*. Table S3 (supplementary) summarises the data obtained for DNA and RNA bases isolated by hydrolysis.

Table 1 also shows a number of changes in the levels of chitin precursors and the current study supports the idea that *y* behaves like a regulatory hormone or cofactor affecting a variety of metabolic pathways. Effects on chitin biosynthesis point to *y* possibly mediating its effects through regulation of ecdysone activity which has been found to regulate genes involved in chitin biosynthesis [18]. It may be that the regulation of chitin biosynthesis is most significant with regard to the function of *y* since formation of the correct chitin structure may be necessary for proper functioning of melanin production.

Acknowledgements

We thank Scottish Universities Life Sciences Alliance for funding for mass spectrometry equipment. Work in the Dow laboratory is generously supported by the UK Biotechnology and Biological Sciences Research Council.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fob.2012.07.007.

References

[1] Lindsley, D.L. and Grell, E.H. (1968) Genetic Variations of *Drosophila melanogaster*, Carnegie Institution of Washington Publication.
[2] Chintapalli, V.R., Wang, J. and Dow, J.A. (2007) Using FlyAtlas to identify better *Drosophila melanogaster* models of human disease. Nat. Genet 39, 715–720.
[3] Foret, S., Kucharski, R., Pittelkow, Y., Lockett, G.A. and Maleszka, R. (2009) Epigenetic regulation of the honeybee transcriptome: unravelling the nature of methylated genes. BMC Genomics 10, 472.
[4] Riedel, F., Vorkel, D. and Eaton, S. (2011) Megalin-dependent yellow endocytosis restricts melanization in the Drosophila cuticle. Development 138, 149–158.
[5] Drapeau, M.D. (2003) A novel hypothesis on the biochemical role of the Drosophila Yellow protein. Biochem. Biophys. Res. Commun 311, 1–3.
[6] Drapeau, M.D., Cyran, S.A., Viering, M.M., Geyer, P.K. and Long, A.D. (2006) A cis-regulatory sequence within the yellow locus of *Drosophila melanogaster* required for normal male mating success. Genetics 172, 1009–1030.
[7] Ferguson, L.C., Green, J., Surridge, A. and Jiggins, C.D. (2011) Evolution of the insect yellow gene family. Mol. Biol. Evol 28, 257–272.
[8] Han, Q., Fang, J., Ding, H., Johnson, J.K., Christensen, B.M. and Li, J. (2002) Identification of *Drosophila melanogaster* yellow-f and yellow-f2 proteins as dopachrome-conversion enzymes. Biochem. J 368, 333–340.
[9] Kamleh, M.A., Hobani, Y., Dow, J.A. and Watson, D.G. (2008) Metabolomic profiling of Drosophila using liquid chromatography Fourier transform mass spectrometry. FEBS Lett 582, 2916–2922.
[10] Kamleh, M.A., Hobani, Y., Dow, J.A., Zheng, L. and Watson, D.G. (2009) Towards a platform for the metabonomic profiling of different strains of *Drosophila melanogaster* using liquid chromatography-Fourier transform mass spectrometry. FEBS J 276, 6798–6809.
[11] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press.
[12] Kriaucionis, S. and Heintz, N. (2009) The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 324, 929–930.
[13] Ni, J.Q., Yuan, X.L., Gu, J.Y., Yue, X.Z., Gu, X.R., Nagaraj, R.H., Crab, J.W. and Clinical Genomic Proteomic (2009) Plasma protein pentosidine and carboxymethyllysine, biomarkers for age-related macular degeneration. Mol. Cell. Proteomics 8, 1921–1933.
[14] Cakouros, D., Mills, K., Denton, D., Paterson, A., Daish, T. and Kumar, S. (2008) dLKR/SDH regulates hormone-mediated histone arginine methylation and transcription of cell death genes. J. Cell Biol 182, 481–495.
[15] Rahman, I., Marwick, J. and Kirkham, P. (2004) Redox modulation of chromatin remodeling: impact on histone acetylation and deacetylation, NF-kappaB and pro-inflammatory gene expression. Biochem. Pharmacol 68, 1255–1267.
[16] Zee, B.M., Levin, R.S., Dimaggio, P.A. and Garcia, B.A. (2010) Global turnover of histone post-translational modifications and variants in human cells. Epigenetics Chromatin 3, 22.
[17] Kucharski, R., Maleszka, J., Foret, S. and Maleszka, R. (2008) Nutritional control of reproductive status in honeybees via DNA methylation. Science 319, 1827–1830.
[18] Yao, Q., Zhang, D., Tang, B., Chen, J., Lu, L. and Zhang, W. (2010) Identification of 20-hydroxyecdysone late-response genes in the chitin biosynthesis pathway. PLoS One 5, e14058.