© 2017. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2017) 220, 1187-1191 doi:10.1242/jeb.150805

SHORT COMMUNICATION

Central metabolic sensing remotely controls nutrient-sensitive endocrine response in *Drosophila* via Sir2/Sirt1–upd2–IIS axis

Kushal K. Banerjee, Rujuta S. Deshpande, Pranavi Koppula, Champakali Ayyub and Ullas Kolthur-Seetharam*

ABSTRACT

Endocrine signaling is central in coupling organismal nutrient status with maintenance of systemic metabolic homeostasis. While local nutrient sensing within the insulinogenic tissue is well studied, distant mechanisms that relay organismal nutrient status in controlling metabolic–endocrine signaling are less well understood. Here, we report a novel mechanism underlying the distant regulation of the metabolic endocrine response in *Drosophila melanogaster*. We show that the communication between the fat body and insulin-producing cells (IPCs), important for the secretion of *Drosophila* insulin-like peptides (dILPs), is regulated by the master metabolic sensor Sir2/Sirt1. This communication involves a fat body-specific direct regulation of the JAK/STAT cytokine upd2 by Sir2/Sirt1. We have also uncovered the importance of this regulation in coupling nutrient inputs with dILP secretion, and distantly controlling insulin/IGF signaling (IIS) in the intestine. Our results provide fundamental mechanistic insights into the top-down control involving tissues that play key roles in metabolic sensing, endocrine signaling and nutrient uptake.

KEY WORDS: Metabolic homeostasis, NAD$^+$ sensor, JAK/STAT ligand, Insulin secretion, Metabolism, Nutrient sensing, DILP secretion, Insulin signaling, Intestine

INTRODUCTION

Metabolic homeostasis is indispensable for all organisms and involves the coupling of metabolic sensing with adaptive responses. In multicellular organisms, metabolic homeostasis depends on efficient communication across diverse organ systems, predominantly by endocrine mechanisms (Frühbeck et al., 2001; Pedersen, 2011; Stefan and Häring, 2013; Unger et al., 1978). Pancreatic hormones such as insulin and glucagon couple organismal nutrient status with nutrient uptake across organ systems (Unger et al., 1978). A large body of work has facilitated our understanding of the mechanisms within insulinogenic cells (specifically, pancreatic β-islets in vertebrates) that mediate the integration of organismal nutrient status with insulin secretion (Rorsman and Braun, 2013). However, emerging findings from evolutionarily diverse organisms highlight the importance of such an endocrine control from distant tissues (Song et al., 2014; Géminard et al., 2009). In this context, relatively less is known about molecular factors that mediate distant regulation of insulin secretion across species.

The evolutionarily conserved NAD$^+$ sensor Sir2/Sirt1 plays critical roles in controlling insulin secretion from the β-islets in the pancreas (Ramachandran et al., 2011; Bordone et al., 2006). Previous reports from our lab and others have indicated a possible role for Sir2/Sirt1 in distant tissues in regulating insulin production and secretion (Palu and Thummel, 2016; Schenk et al., 2011; Wang et al., 2011; Purushotham et al., 2009; Banerjee et al., 2013). However, the mechanistic details and the physiological understanding of this endocrine control are currently lacking.

Drosophila melanogaster has been extensively used to investigate the physiological and genetic bases of metabolic homeostasis. Under conditions of nutrient excess, the insulin-producing cells (IPCs) located in the median neurosecretory cluster (mNSC) in the brain secrete *Drosophila* insulin-like peptides (dILPs) (Ikeya et al., 2002; Kim and Rulifson, 2004). Interestingly, IPCs lack the ability to sense organismal metabolic status and depend on signals from the fat body (Ikeya et al., 2002; Kim and Rulifson, 2004). Genetic evidence implicates the metabolic transcription factor *FOXO* (dFOXO) (Hwangbo et al., 2004), target of rapamycin (TOR) signaling (Géminard et al., 2009) and a secretory cytokine *upd2* (Rajan and Perrimon, 2012), among others, in mediating the distant control of dILP secretion by the fat body. However, the physiological relevance of these factors and a role for a master metabolic sensor within the fat body in establishing systemic metabolic homeostasis by controlling an inter-organ communication network has not been addressed.

Here, we have investigated the molecular underpinnings of the distant control of a metabolic response by addressing the role of the master metabolic sensor *Sir2/Sirt1* within the fat body in regulating an endocrine response to nutrient fluctuations.

MATERIALS AND METHODS

Fly stocks

$S_1$106 (P{Switch 1}106 Gal4), Sir2/Sirt1^{EP2300} (w^{1118}; P{w[+mC] =EP}Sirt1[EP2300] DnaJ-H[EP2300]/CyO), chico (cn^1 P{ry11} chico^1/CyO; ry^{506}) and InR (InR^{E19}/TM2) stocks were obtained from Bloomington Stock Center (Indiana University). SIR2^{RNAi} (23201) and upd2^{RNAi} (6513) lines were obtained from Vienna *Drosophila* RNAi Center (VDRC).

Growth conditions

Flies were grown on standard CM/S/Y media (8.6% cornmeal, 5% sucrose, 2.5% yeast) under non-crowding conditions at 25°C with a 12 h:12 h light:dark cycle. Age-matched 3–5 day old female flies were used for all analyses.

Activation of Gal4

Gene-switch Gal4 *$S_1$106* was crossed with relevant transgene lines. Gal4 was activated to drive UAS-RNAi or EP-Sir2/Sirt1 lines, by rearing the appropriate lines on diets supplemented with 200 µmol l^{-1} (or 400 µmol l^{-1}, in the case of RNAi combinations) RU486 (Mifeprestone, Sigma-Aldrich; hereafter, RU) dissolved in 95% ethanol. Flies that were reared on diets containing only ethanol served as controls.

Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Colaba, Mumbai 400 005, India.

*Author for correspondence (ullas@tifr.res.in)

U.K., 0000-0003-2612-1743

Received 20 October 2016; Accepted 12 January 2017

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 1187-1191 doi:10.1242/jeb.150805

Glucose stimulation

Flies grown on standard diet were fasted for 16 h on 2% agar. Glucose stimulation was performed by shifting these flies to vials containing 10% glucose media for 1 h. Immediately after glucose stimulation, the flies were collected for hemolymph extraction.

Hemolymph isolation and dILP5 quantification

Hemolymph was isolated from the head capsule of 50 flies by capillary action as described earlier (Banerjee et al., 2013). For western blot analysis, protein lysates were prepared by incubating the hemolymph extracts in lysis buffer. The protein content of the hemolymph lysates was estimated by loading 25 µl of the extract on an SDS-PAGE gel and normalized to 25 µg of bovine serum albumin (BSA) by Coomassie Brilliant Blue (R250) staining. Following this, 1 mg of total hemolymph protein extract was loaded on a separate gel and proteins below 25 kDa were probed with anti-dILP5 antibody (ab128220, Abcam) to quantify circulating dILP5. The upper part of the same gel was stained with Coomassie Brilliant Blue and used as a loading control.

Tissue dissections

Adult flies were anesthetized using CO_2 and the intestines/fat body were dissected in ice-cold Tris-buffered saline (TBS; pH 7.4). Fat body/intestines were pooled for RNA isolation (in 1 ml Trizol) or protein lysates (in 200 µl RIPA), respectively, to generate individual replicates.

RNA isolation and reverse transcription

RNA was isolated using Trizol (15596018, Life Technologies) as per the manufacturer's instructions. For cDNA synthesis, 2 µg of RNA was reverse transcribed using SuperScript-III reverse transcriptase kit (18080-044, Life technologies).

Real-time PCR analysis

Real-time qPCR (qRT-PCR) reactions to quantify relative mRNA expression were performed using Kapa SYBR green (KK4600, KAPA Biosystems) on Eppendorf Realplex. Data were analyzed by the ΔΔCT method. Triplicate samples (30 flies each) from at least two separate experiments were used. *rp49* and *actin5c* were used as normalization controls. The primer pairs used for the qRT-PCR are as follows; *upd2:* forward CGGAACATCACGATGAGCGAAT and reverse TCGGCAGGAACTTGTACTCG, *actin5c*: forward GAGGCTTGCGGCATCCACGAGACCAC and reverse GACAG-AGTACTTGCGCTCTGGCG and *rp49*: forward TACAGGCCC-AAGATCGTGAA and reverse TCTCCTTGCGCTTCTTGGA. The cycling conditions were as prescribed by the manufacturers.

Western blotting

Protein lysates were prepared by incubating tissues on ice for 15 min in RIPA buffer (50 mmol l^{-1} Tris-HCl pH 7.5, 150 mmol l^{-1} NaCl, 1 mmol l^{-1} EDTA, 6 mmol l^{-1} EGTA, 20 mmol l^{-1} NaF, 1% Triton X-100) with protease inhibitors (05-056-489001, Roche) and phosphatase inhibitor (PhosSTOP, 04-906-837-001, Roche). Anti-pAKT (1:750, 9271, Cell Signaling Technology), anti-AKT (1:750, 9272, Cell Signaling Technology) and anti-actin (1:3000, A1978, Sigma Aldrich) antibodies were used for the western blots. Chemiluminescence detection (1859023/185022, Thermo Scientific) was used to visualize the bands.

Statistical analyses

SigmaPlot12.0 was used for statistical analyses. Student's *t*-test and ANOVA were used to determine statistical significance of the data.

RESULTS AND DISCUSSION

Fat body *Sir2*/*Sirt1* distantly controls dILP5 secretion from the IPCs

Clinical evidence implicates impaired metabolic sensing by central metabolic organs, such as the liver and adipocytes, in metabolic syndromes such as type 2 diabetes (T2DM) and insulin resistance (Song et al., 2014; Roden, 2006; Guilherme et al., 2008). In this regard, hepatic and adipocyte-specific disruption of Sirt1 results in hyperinsulinemia in mammals (Purushotham et al., 2009; Wang et al., 2011; Gillum et al., 2011), but the mechanisms are poorly understood. Although a role for *Sir2/Sirt1* in controlling dILP2 secretion was identified (Palu and Thummel, 2016), the tissue of origin of *Sir2/Sirt1*-dependent dILP control is still unknown.

To address the link between central metabolic sensing and an endocrine metabolic response, we investigated the role of fat body *Sir2/Sirt1* in controlling the secretion of dILP from the IPCs. Knockdown of *Sir2/Sirt1* in the fat body (S_1106;$Sir2^{RNAi}$ with and without RU) (Osterwalder et al., 2001) significantly increased hemolymph dILP5 levels (Fig. 1A), while over-expression (S_1106/$Sir2^{OE}$ with and without RU) reduced circulating dILP5 to undetectable levels (Fig. 1B). Acute glucose administration to control flies led to an increase in dILP5 secretion in the hemolymph (Fig. 1C). Furthermore, fat body-specific *Sir2/Sirt1* knockdown resulted in heightened secretion of dILP5 and prevented a further increase in dILP5 secretion upon administration of glucose (Fig. 1C).

These results clearly demonstrate a role for *Sir2/Sirt1* in the *Drosophila* fat body in the secretion of dILP5 from IPCs. More importantly, these findings provided a premise to interrogate the mechanistic underpinnings of the role of *Sir2/Sirt1* in mediating inter-tissue communication between central metabolic tissue and insulinogenic tissue.

Fat body *Sir2*/*Sirt1* controls *upd2* expression to regulate dILP5 secretion from the IPCs

Fat body, liver and adipocytes have well-established and evolutionarily conserved endocrine functions (Frühbeck et al.,

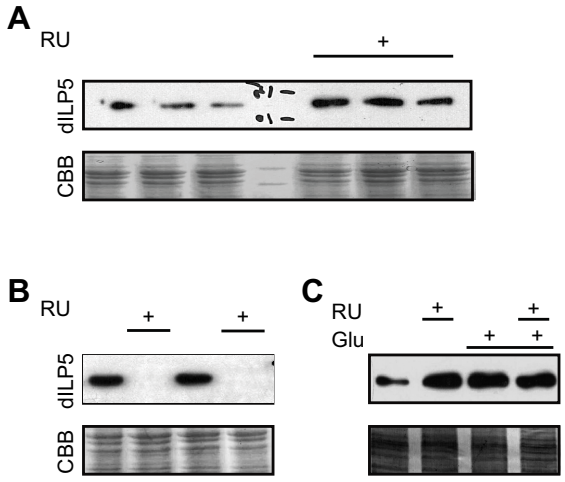

Fig. 1. Fat body Sir2/Sirt1 distantly controls the secretion of *Drosophila* insulin-like peptide 5 (dILP5) from insulin-producing cells (IPCs). Western blots of dILP5 in the hemolymph in (A) fbSir2^{RNAi} flies ($S_1106;Sir2^{RNAi}$ ±RU), (B) fbSir2^{OE} flies ($S_1106/Sir2^{OE}$ ±RU) and (C) fbSir2^{RNAi} flies ($S_1106;Sir2^{RNAi}$ ±RU) stimulated with 10% glucose (Glu). Coomassie Brilliant Blue (CBB)-stained gels were used as loading controls for hemolymph protein extracts (see Materials and methods). In the figure and text, Sirt1^{EP2300} is represented as Sir2^{OE}.

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 1187-1191 doi:10.1242/jeb.150805

2001; Ouchi et al., 2011; Stefan and Häring, 2013). However, insights into molecular mechanisms within these tissues that couple metabolic inputs to the regulation of cytokines (adipokines/hepatokines) are limited.

The JAK/STAT ligand *upd2* is a humoral link between the fat body and IPCs, which controls dILP secretion, specifically in response to glucose feeding (Rajan and Perrimon, 2012). To gain insights into the mechanisms by which fat body Sir2/Sirt1 might remotely regulate dILP5 secretion from the IPCs, we investigated its genetic interaction with *upd2*. As reported earlier (Rajan and Perrimon, 2012), knockdown of *upd2* in the fat body (S_1106; $upd2^{RNAi}$ with and without RU) reduced circulating dILP5 levels (Fig. 2A). Furthermore, simultaneous knockdown of *upd2* and *Sir2/Sirt1* within the fat body (S_1106;$Sir2^{RNAi}$/$upd2^{RNAi}$ with and without RU; Fig. 2B) resulted in undetectable circulatory dILP5, mimicking S_1106;$upd2^{RNAi}$ flies. These results pointed towards an epistatic association between *Sir2/Sir1* and *upd2* in regulating dILP5. Physiologically, the ability to regulate dILP5 secretion suggested a role for *upd2* in controlling a response to nutrient inputs. In this regard, we knocked down *upd2* in the fat body and assayed for glucose sensitivity. We found that knockdown of *upd2* in the fat body blunted the glucose-stimulated increase in hemolymph dILP5 levels (Fig. 2C). Importantly, the combined knockdown of *upd2* and *Sir2/Sirt1* in the fat body subdued the glucose-dependent increase in dILP5 secretion (Fig. 2D). The effects of combinatorial knockdown of *upd2* and *Sir2/Sirt1* in the fat body on dILP5 secretion under basal as well as glucose-stimulated conditions indicated an epistatic interaction between these two factors in the regulation of glucose sensitivity (Fig. 2). Motivated by our results, we investigated the nature of the interaction between *Sir2/Sirt1* and *upd2*.

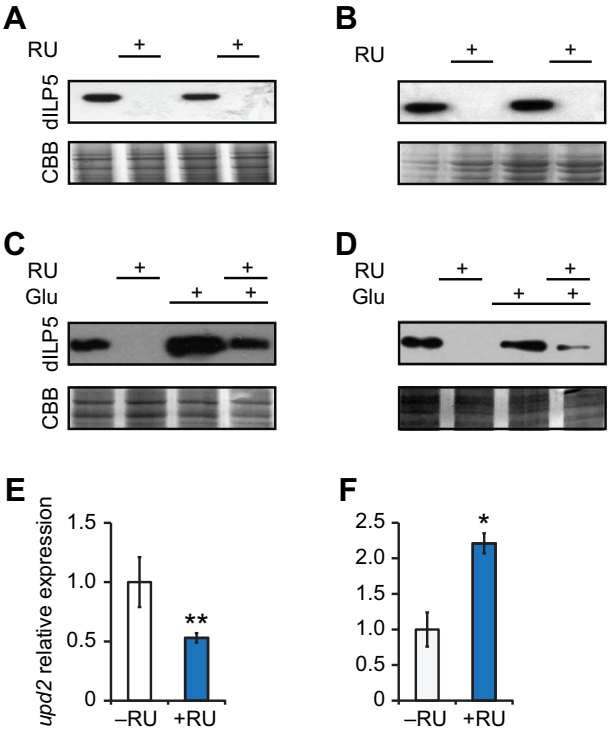

Fig. 2. Direct interplay between Sir2/Sirt1 and upd2 mechanistically underpins the long-range control of dILP5 secretion by the fat body. (A,B) Western blots of dILP5 in the hemolymph of (A) fbupd2^{RNAi} flies (S_1106; $upd2^{RNAi}$ ±RU) and (B) flies with a simultaneous knockdown of fbSir2 and fbupd2 (S_1106;$Sir2^{RNAi}$/$upd2^{RNAi}$ ±RU). (C,D) Western blots of dILP5 after 10% glucose stimulation in hemolymph of (C) fbupd2^{RNAi} flies (S_1106; $upd2^{RNAi}$ ±RU) and (D) flies with a simultaneous knockdown of fbSir2 and fbupd2 (S_1106;$Sir2^{RNAi}$/$upd2^{RNAi}$ ±RU). (E,F) mRNA expression analyses of upd2 in the fat body of (E) fbSir2^{OE} (S_1106/$Sir2^{OE}$ ±RU) flies and (F) fbSir2^{RNAi} flies (S_1106;$Sir2^{RNAi}$ ±RU). Coomassie Brilliant Blue (CBB)-stained gels were used as loading controls for hemolymph protein extracts (see Materials and methods). In the figure and text, $Sirt1^{EP2300}$ is represented as $Sir2^{OE}$. Data in E and F are shown as means±s.e.m. Student's *t*-test was used for statistical analyses: **P*<0.05, ***P*<0.01. Representative results from two separate experiments with triplicate samples from fat body (*n*=10×3) were used for qPCR.

As shown in Fig. 2, overexpression of *Sir2/Sirt1* significantly reduced *upd2* expression (Fig. 2E) and knockdown of *Sir2/Sirt1* in the fat body led to a robust increase in the expression of *upd2* (Fig. 2F). These results indicated that in addition to exhibiting an epistatic association in physiologically controlling dILP5 secretion in response to glucose inputs, *Sir2/Sirt1* acts as an upstream regulator of *upd2* expression in the fat body (Fig. 2E,F). The regulation of *upd2* by *Sir2/Sirt1* in the fat body provides comprehensive evidence for the control of a JAK/STAT ligand by *Sir2/Sirt1* in metabolic signaling. While Sirt1 has previously been shown to regulate Stat3 in the control of hepatic gluconeogenesis (Nie et al., 2009), our results show for the first time the importance of *Sir2/Sirt1*-dependent control of JAK/STAT signaling in insulin secretion.

Sir2/Sirt1-upd2 interplay in the fat body distantly controls ligand-dependent intestinal insulin signaling

Clinical reports correlate dysfunctional central metabolic sensing by liver and brain with metabolic maladaptation in the intestine (Badman and Flier, 2005). However, whether metabolic sensing within central metabolic organs can influence metabolic signaling pathways, such as insulin/IGF signaling (IIS) in the intestine remains elusive.

Inspired by our results, we sought to investigate the role of the interplay between *Sir2/Sirt1* and *upd2* in controlling intestinal IIS. Knockdown of *Sir2/Sirt1* in the fat body led to an increase in intestinal insulin signaling (Fig. 3A), while overexpression of *Sir2/Sirt1* in the fat body resulted in a strong reduction in insulin signaling within the intestine (Fig. 3B). These results provide the first evidence to implicate the role of a metabolic sensor in liver/adipocytes in distantly controlling intestinal IIS.

In vivo and *in vitro* studies have elucidated that systemic insulin signaling is controlled by ligand (insulin)-dependent and -independent mechanisms (Pessin and Saltiel, 2000). In keeping with this, we wanted to ascertain whether the distant control of intestinal insulin signaling by fat body *Sir2/Sirt1* involved a ligand (dILP5, in this case)-independent or -dependent mechanism. In this regard, we reasoned that if fat body *Sir2/Sirt1*-dependent control of intestinal insulin signaling is ligand (dILP5) independent, then a reduction in insulin signaling via genetic disruption would obviate this coupling. Conversely, retention of the sensitivity of intestinal insulin signaling to changes in fat body *Sir2/Sirt1* levels despite the genetic ablation of insulin signaling would suggest a ligand-dependent mechanism. We employed two models of reduced insulin signaling: (1) heterozygosity of *InR* (Tatar et al., 2001) and (2) heterozygosity of *chico* (Clancy et al., 2001). Heterozygosity of *InR* ($S_1106/+$;$Sir2^{RNAi}$/*InR* without RU) and *chico* ($S_1106/chico$; $Sir2^{RNAi}/+$ without RU) attenuated intestinal insulin signaling (Fig. 3C,D, lanes 7–9) basally. Interestingly, simultaneous knockdown of *Sir2/Sirt1* in the fat body of the *InR* ($S_1106/+$; $Sir2^{RNAi}$/*InR* with RU) and *chico* ($S_1106/chico$;$Sir2^{RNAi}/+$ with RU) heterozygotes resulted in an increase in pAKT levels (Fig. 3C,D, lanes 10–12) when compared with those exhibited by only the heterozygote background (Fig. 3C,D, lanes 7–9).

Journal of Experimental Biology (2017) 220, 1187-1191 doi:10.1242/jeb.150805

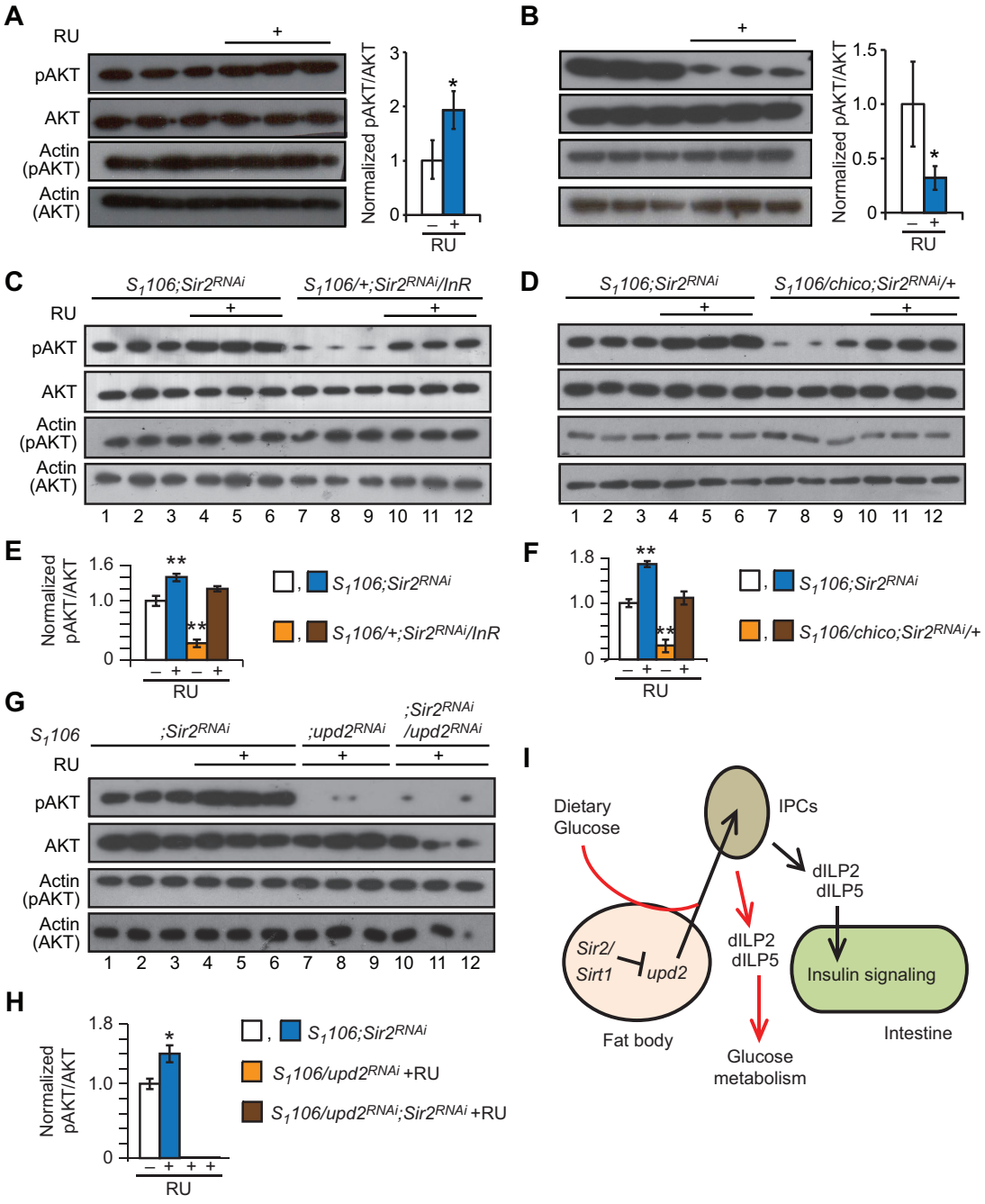

Fig. 3. Genetic epistasis implicates fat body Sir2/Sirt1–upd2 interplay in the remote regulation of intestinal insulin signaling. (A,B) Western blot of pAKT and AKT in the intestine of (A) fbSir2^{RNAi} flies ($S_1106;Sir2^{RNAi}$ ±RU) and (B) fbSir2^{OE} ($S_1106/Sir2^{OE}$ ±RU). (C,D) Intestinal pAKT and AKT levels in control and fbSir2^{RNAi} ($S_1106;Sir2^{RNAi}$ ±RU: lanes 1–6), (C) InR heterozygotes ($S_1106/+;Sir2^{RNAi}/InR$ without RU: lanes 7–9), (D) chico heterozygotes ($S_1106/chico;Sir2^{RNAi}/+$ without RU: lanes 7–9), (C) InR heterozygotes with a simultaneous knockdown of fbSir2 ($S_1106/+;Sir2^{RNAi}/InR$ with RU: lanes 10–12) and (D) chico heterozygotes with a simultaneous knockdown of fbSir2 ($S_1106/chico;Sir2^{RNAi}/+$ with RU: lanes 10–12). (E,F) Densitometric quantification of western blots in C and D, respectively. (G) Intestinal pAKT and AKT levels in control and fbSir2^{RNAi} ($S_1106;Sir2^{RNAi}$ ±RU: lanes 1–6) and fbupd2^{RNAi} flies ($S_1106/upd2^{RNAi}$ +RU: lanes 7–9), and in flies with a simultaneous knockdown of fbSir2 and fbupd2 ($S_1106/upd2^{RNAi};Sir2^{RNAi}$ +RU: lanes 10–12). (H) Normalized densitometric quantification of two independent replicate experiments including the western blot shown in G. (I) *Sir2*/*Sirt1*-dependent regulation of the expression of *upd2* within the fat body maintains a nutrient-sensitive endocrine response by coupling nutrient inputs (from glucose) to dILP2/dILP5 secretion from the IPCs and insulin signaling in the intestine. In the figure and text, $Sirt1^{EP2300}$ is represented as $Sir2^{OE}$. A 175 µg sample of total protein isolated from pooled fly intestines (n=10 per sample) was analyzed. Data in A, B, E, F and H are shown as means±s.e.m. Student's t-test and two-way ANOVA were used for statistical analyses: *P<0.05, **P<0.01.

Given the complex interplay between dILPs (Grönke et al., 2010; Okamoto and Nishimura, 2015), we knocked down the upstream regulator *upd2* simultaneously with *Sir2/Sirt1* in the fat body to gain mechanistic insights into intestinal control of IIS. Knockdown of *upd2* alone in the fat body ($S_1106;upd2^{RNAi}$ with and without RU) or with a simultaneous knockdown of *Sir2/Sirt1* ($S_1106;Sir2^{RNAi}/upd2^{RNAi}$ with and without RU) was sufficient to bring about a strong reduction in intestinal insulin signaling (Fig. 3G, lanes 7–12) and override the increase observed in response to fat body *Sir2/Sirt1* knockdown (Fig. 3G, lanes 1–6).

Journal of Experimental Biology

In summary, we have provided mechanistic and physiological insights into the regulation of dILP5 secretion by the fat body involving the NAD^+-dependent metabolic sensor *Sir2/Sirt1* and the JAK/STAT ligand *upd2*. A recent study reported that *Sir2/Sirt1* is a negative regulator of dILP2 secretion (Palu and Thummel, 2016). However, whether this is mediated in a tissue-autonomous or -non-autonomous manner was unclear. A genetic or molecular interaction of *Sir2/Sirt1* with previously established cytokine communicators that may bring about such a control has also not been demonstrated. In this context, we emphasize that this is the first report of an inter-tissue communication between the fat body, IPCs and the intestine, which is controlled by the NAD^+-dependent *Sirt1/Sir2*. Specifically, our findings provide conclusive mechanistic underpinnings of a fat-body *Sir2/Sirt1*-mediated distant regulation of dILP5 via *upd2*. These results support the role of a central metabolic organ in controlling intestinal metabolic signaling and highlight the importance of a master metabolic sensor in this distant control of organismal physiology (Fig. 3I). Given the recent advances towards understanding a distant control of insulin secretion in vertebrates and invertebrates, our study identifies an axis between nutrient sensing by the fat body and humoral inputs from the neuroendocrine system.

Acknowledgements
We thank Ms Sweta Parik and Ms Namrata Shukla for help in preparing the manuscript and for their critical inputs.

Competing interests
The authors declare no competing or financial interests.

Author contributions
K.K.B., R.S.D. and P.K. performed experiments. K.K.B., C.A. and U.K.-S. designed experiments. K.K.B., C.A. and U.K.-S. wrote the manuscript.

Funding
We acknowledge funding received from Department of Atomic Energy/Tata Institute of Fundamental Research (DAE/TIFR), Government of India.

References
Badman, M. K. and Flier, J. S. (2005). The gut and energy balance: visceral allies in the obesity wars. *Science* **307**, 1909-1914.
Banerjee, K. K., Ayyub, C., Sengupta, S. and Kolthur-Seetharam, U. (2013). Fat body dSir2 regulates muscle mitochondrial physiology and energy homeostasis nonautonomously and mimics the autonomous functions of dSir2 in muscles. *Mol. Cell. Biol.* **33**, 252-264.
Bordone, L., Motta, M. C., Picard, F., Robinson, A., Jhala, U. S., Apfeld, J., Mcdonagh, T., Lemieux, M., Mcburney, M., Szilvasi, A. et al. (2006). Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic beta cells. *PLoS Biol.* **4**, e31.
Clancy, D. J., Gems, D., Harshman, L. G., Oldham, S., Stocker, H., Hafen, E., Leevers, S. J. and Partridge, L. (2001). Extension of life-span by loss of CHICO, a Drosophila insulin receptor substrate protein. *Science* **292**, 104-106.
Frühbeck, G., Gómez-Ambrosi, J., Muruzábal, F. J. and Burrell, M. A. (2001). The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am. J. Physiol. Endocrinol. Metab.* **280**, E827-E847.
Géminard, C., Rulifson, E. J. and Léopold, P. (2009). Remote control of insulin secretion by fat cells in drosophila. *Cell Metab.* **10**, 199-207.
Gillum, M. P., Kotas, M. E., Erion, D. M., Kursawe, R., Chatterjee, P., Nead, K. T., Muise, E. S., Hsiao, J. J., Frederick, D. W., Yonemitsu, S. et al. (2011). SirT1 regulates adipose tissue inflammation. *Diabetes* **60**, 3235-3245.
Grönke, S., Clarke, D.-F., Broughton, S., Andrews, T. D. and Partridge, L. (2010). Molecular evolution and functional characterization of Drosophila insulin-like peptides. *PLoS Genet.* **6**, p.e1000857.
Guilherme, A., Virbasius, J. V., Puri, V. and Czech, M. P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.* **9**, 367-377.
Hwangbo, D. S. Gersham, B., Tu, M.-P., Palmer, M. and Tatar, M. (2004). Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature* **429**, 562-566.
Ikeya, T., Galic, M., Belawat, P., Nairz, K. and Hafen, E. (2002). Nutrient-Dependent Expression of Insulin-like Peptides from Neuroendocrine Cells in the CNS Contributes to Growth Regulation in Drosophila. *Curr. Biol.* **12**, 1293-1300.
Kim, S. K. and Rulifson, E. J. (2004). Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. *Nature* **431**, 316-320.
Nie, Y., Erion, D. M., Yuan, Z., Dietrich, M., Shulman, G. I., Horvath, T. L. and Gao, Q. (2009). STAT3 inhibition of gluconeogenesis is downregulated by SirT1. *Nat. Cell Biol.* **11**, 492-500.
Okamoto, N. and Nishimura, T. (2015). Signaling from glia and cholinergic neurons controls nutrient-dependent production of an insulin-like peptide for drosophila body growth. *Dev. Cell* **35**, 295-310.
Osterwalder, T., Yoon, K. S., White, B. H. and Keshishian, H. (2001). A conditional tissue-specific transgene expression system using inducible GAL4. *Proc. Natl. Acad. Sci. USA* **98**, 12596-12601.
Ouchi, N., Parker, J. L., Lugus, J. J. and Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **11**, 85-97.
Palu, R. A. S. and Thummel, C. S. (2016). Sir2 acts through hepatocyte nuclear factor 4 to maintain insulin signaling and metabolic homeostasis in drosophila. *PLoS Genet.* **12**, e1005978.
Pedersen, B. K. (2011). Muscles and their myokines. *J. Exp. Biol.* **214**, 337-346.
Pessin, J. E. and Saltiel, A. R. (2000). Signaling pathways in insulin action: molecular targets of insulin resistance. *J. Clin. Invest.* **106**, 165-169.
Purushotham, A., Schug, T. T., Xu, Q., Surapureddi, S., Guo, X. and Li, X. (2009). Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. *Cell Metab.* **9**, 327-338.
Rajan, A. and Perrimon, N. (2012). Drosophila cytokine unpaired 2 regulates physiological homeostasis by remotely controlling insulin secretion. *Cell* **151**, 123-137.
Ramachandran, D., Roy, U., Garg, S., Ghosh, S., Pathak, S. and Kolthur-Seetharam, U. (2011). Sirt1 and mir-9 expression is regulated during glucose-stimulated insulin secretion in pancreatic β-islets. *FEBS J.* **278**, 1167-1174.
Roden, M. (2006). Mechanisms of Disease: hepatic steatosis in type 2 diabetes—pathogenesis and clinical relevance. *Nature Clinical Practice Endocrinology & Metabolism* **2**, 335-348.
Rorsman, P. and Braun, M. (2013). Regulation of insulin secretion in human pancreatic islets. *Annu. Rev. Physiol.* **75**, 155-179.
Schenk, S., Mccurdy, C. E., Philp, A., Chen, M. Z., Holliday, M. J., Bandyopadhyay, G. K., Osborn, O., Baar, K. and Olefsky, J. M. (2011). Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. *J. Clin. Invest.* **121**, 4281-4288.
Song, W.-J. Mondal, P., Wolfe, A., Alonso, L. C., Stamateris, R., Ong, B. W. T., Lim, O. C., Yang, K. S., Radovick, S., Novaira, H. J. et al. (2014). Glucagon regulates hepatic kisspeptin to impair insulin secretion. *Cell Metab.* **19**, 667-681.
Stefan, N. and Häring, H.-U. (2013). The role of hepatokines in metabolism. *Nat. Rev. Endocrinol.* **9**, 144-152.
Tatar, M., Kopelman, A., Epstein, D., Tu, M. P., Yin, C. M. and Garofalo, R. S. (2001). A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107-110.
Unger, R. H., Dobbs, R. E. and Orci, L. (1978). Insulin, glucagon, and somatostatin secretion in the regulation of metabolism. *Annu. Rev. Physiol.* **40**, 307-343.
Wang, R.-H., Kim, H.-S., Xiao, C., Xu, X., Gavrilova, O. and Deng, C.-X. (2011). Hepatic Sirt1 deficiency in mice impairs mTorc2/Akt signaling and results in hyperglycemia, oxidative damage, and insulin resistance. *J. Clin. Invest.* **121**, 4477-4490.