Drosophila protein phosphatase V regulates lipid homeostasis via the AMPK pathway

Dear Editor,

Protein phosphorylation is essential for multiple cellular processes. This post-translational modification is regulated by kinase-mediated phosphorylation and phosphatase-mediated dephosphorylation. Drosophila protein phosphatase V (PpV) and Saccharomyces cerevisiae Sit4 are homologs of mammalian PP6, which belongs to the PP2A subfamily of serine/threonine phosphatases (Mann et al., 1993; Wang et al., 2012). In addition to the catalytic subunit, PP6 holoenzyme also contains a regulatory subunit (PP6R1, PP6R2, or PP6R3 in human; Sap4, Sap155, Sap185, or Sap190 in yeast; CG10289 in fly) and a scaffold subunit (Morales-Johansson et al., 2009). Studies in S. cerevisiae showed that Sit4 knockout strain had reduced lipid droplet content and increased phosphorylation of SNF1 (a homolog of mammalian and Drosophila AMPK) at an evolutionally conserved activation site (Bozaquel-Morais et al., 2010; Ruiz et al., 2011). However, its function in metazoan has not been well explored.

To investigate the physiological role of PpV, we ubiquitously knocked down its regulatory or catalytic subunit with transgenic RNAi flies (PpVR-IR or PpVc-IR). Quantitative real-time PCR (qRT–PCR) analyses indicated 60%–75% knockdown efficiency for either gene in larvae (data not shown). Knockdown of PpVR did not show obvious changes in larval body size, but caused lethality at pupal stage. On the contrary, knockdown of PpVc resulted in severely reduced body size and larval lethality (Figure 1A). Triacylglyceride (TAG) is a main form for nutritional storage in Drosophila larvae. Knockdown of PpVc, but not PpVR, resulted in significantly reduced TAG levels in these larvae (Figure 1B).

The AMPK pathway inhibits adipocyte differentiation and lipogenesis while promotes lipid mobilization, thus playing a key role in lipid catabolism (Viollet and Andreelli, 2011; Hardie et al., 2012). It has been shown that Sit4 contributes to the regulation of SNF1/AMPK activities in yeast (Bozaquel-Morais et al., 2010; Ruiz et al., 2011). We examined levels of AMPK phosphorylated at a conserved Thr-172 site (representing the active form of AMPK) with a phospho-specific antibody. Ubiquitous knockdown of either PpVR or PpVc caused a clear increase in AMPK phosphorylation levels in larvae (Figure 1C). The PpVc RNAi showed a stronger effect, consistent with the severer phenotype. We could not monitor the total AMPK levels due to lack of antibodies that recognize Drosophila AMPK. However, the mRNA levels of AMPK were not increased by PpVR or PpVc knockdown (Supplementary Figure S1A), suggesting that the elevated AMPK phosphorylation level did not result from upregulated AMPK expression.

Drosophila fat body is functionally equivalent to mammalian white adipose tissue and liver, and is the main storage site for TAG. It also communicates with other organs and regulates the growth of whole body (Rajan and Perrimon, 2011). After we knocked down PpVc or PpVR specifically in the fat body with cg-Gal4, the flies were viable through adulthood, with reduced body size (Figure 1D) and weight (Supplementary Figure S1B). Also at the larval stage, we observed reductions in larval TAG levels (Figure 1E) and increases in AMPK phosphorylation levels in the fat body (Figure 1F) by PpVc or PpVR knockdown. To investigate whether these effects were dependent on increased AMPK activation, we expressed PpVc or PpVc dsRNA in the fat body simultaneously with a transgenic RNAi line targeting the AMPK catalytic subunit (AMPK-IR), which did not attenuate the knockdown efficiency of PpVc or PpVR (Supplementary Figure S1C). Knockdown of AMPK significantly rescued the reductions in body size (Figure 1D), body weight (Supplementary Figure S1B), and TAG levels (Figure 1E) caused by PpVc or PpVR knockdown, suggesting that PpV regulates Drosophila lipid metabolism and growth through the AMPK pathway.

We further examined how PpV affected the fat body at cellular level. We expressed dsRNA against PpVc or PpVR in a clonal pattern in the fat body. Compared with surrounding wild-type cells, cells with knockdown of PpVc or PpVR had severely reduced cell size (Figure 1G and Supplementary Figure S2A–C). We also induced PpVR knockdown clones with a P element insertion allele of PpVc (CG10289(22530) and obtained similar results (Figure 1H), excluding the possibility that the observed phenotype by RNAi was due to an off-target effect. Similar to that observed in whole body, simultaneous knockdown of AMPK largely rescued the decrease in cell size by knockdown of PpVc (Figure 1G and Supplementary Figure S2D–F). As the AMPK pathway and the mTOR pathway antagonize to regulate energy homeostasis and cell growth (Inoki et al., 2012), we tested whether expression of a constitutively active S6K (Barcelo and Stewart, 2002), which is a downstream effector of mTOR, could rescue the reduced cell size. Indeed, the effect of PpVc or PpVR on cell size was largely reversed by activated S6K (Figure 1G and Supplementary Figure S2G–I).

Lastly, we tested whether PpV is a specific phosphatase in AMPK activation and lipid homeostasis. CG11597 is another member of PP2A subfamily of serine/threonine phosphatases and has the highest sequence homology to PpV. However, ubiquitous knockdown of CG11597 with a transgenic RNAi line (CG11597-IR) had no effect on AMPK phosphorylation level in larvae (Supplementary Figure S3A). In addition, expression of CG11597 dsRNA in a clonal pattern had no effect on cell size in the fat body (Supplementary Figure S3B–D). Taken together, these results suggest that
Figure 1 PpV regulates lipid homeostasis via the AMPK pathway in Drosophila. (A) The effect on larval body size by ubiquitous knockdown of PpV$_{e}$ or PpV$_{c}$. (B) The effect on larval TAG levels by ubiquitous knockdown of PpV$_{e}$ or PpV$_{c}$. (C) The effect on AMPK phosphorylation levels by ubiquitous knockdown of PpV$_{e}$ or PpV$_{c}$, shown by western blotting (left) and quantification (right). (D) The effect on adult body size by fat body-specific knockdown of PpV, AMPK, or simultaneous knockdown of PpV and AMPK. (E) The effect on larval TAG levels by fat body-specific knockdown of PpV, AMPK, or simultaneous knockdown of PpV and AMPK. (F) The effect on AMPK phosphorylation levels in the fat body by fat body-specific knockdown of PpV$_{e}$ or PpV$_{c}$, shown by western blotting (left) and quantification (right). (G) Quantification of the relative cell size affected by clonal expression of PpV dsRNA, AMPK dsRNA, S6K$_{TE}$, or their combined expression. (H) PpV$_{c}$ knockdown clones were induced in the fat body by FRT-mediated mitotic recombination. PpV$_{c}^{F02530}$/PpV$_{e}^{F02530}$ cells are marked by the lack of GFP (arrows), +/+ (wild-type) cells by the presence of brighter GFP signal due to two copies of GFP (arrowheads), and PpV$_{c}^{F02530}$/+ cells by the presence of weak GFP signal due to one copy of GFP (not indicated). Bar represents mean ± SEM. The two-tailed, unpaired t-test was used. *P<0.05, **P<0.01; ns, not significant. The P-values between each experimental group and the control group are indicated in black. The P-values between two experimental groups are indicated in red.
PpV is a specific regulator of *Drosophila* adipogenesis and acts through the AMPK pathway. Given that AMPK is an attractive therapeutic target in the treatment of cancer and diabetes, our study uncovers a novel mechanism to regulate AMPK and provides additional cues for drug design.

[Supplementary material is available at Journal of Molecular Cell Biology online. We thank Dr Mikael Bjorklund for in-depth discussion. This study was supported by the National Basic Research Program of China (973 Program) (2011CB943900), Hundred Talent Program, and the National Natural Science Foundation of China (31071145, 31371493).]

Dingzi Yin1,†, Ping Huang2,†, Jiarui Wu1,3,*, and Haiyun Song2,*

1Key Laboratory of Systems Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
2Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China
3Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China

†These authors contributed equally to this work.
*Correspondence to: Jiarui Wu, E-mail: wujr@sibs.ac.cn; Haiyun Song, E-mail: hysong@sibs.ac.cn

References


