The role of translationally controlled tumor protein in proliferation of *Drosophila* intestinal stem cells

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Translationally controlled tumor protein (TCTP) is a highly conserved protein functioning in multiple cellular processes, ranging from growth to immune responses. To explore the role of TCTP in tissue maintenance and regeneration, we employed the adult *Drosophila* midgut, where multiple signaling pathways interact to precisely regulate stem cell division for tissue homeostasis. Tctp levels were significantly increased in stem cells and enteroblasts upon tissue damage or activation of the Hippo pathway that promotes regeneration of intestinal epithelium. Stem cells with reduced Tctp levels failed to proliferate during normal tissue homeostasis and regeneration. Mechanistically, Tctp forms a complex with multiple proteins involved in translation and genetically interacts with ribosomal subunits. In addition, Tctp increases both Akt1 protein abundance and phosphorylation in vivo. Altogether, Tctp regulates stem cell proliferation by interacting with key growth regulatory signaling pathways and the translation process in vivo.

TCTP | AKT | Yorkie | translation | regeneration

Translationally controlled tumor protein (TCTP, also known as TPT1) was originally discovered as a protein that was rapidly translated upon serum stimulation in mouse NIH 3T3 and sarcoma ascites cells (1, 2). Subsequently, TCTP has been characterized in a wide range of eukaryotes, including mammals, insects, and plants, and has been linked to multiple biological processes, ranging from growth control to immune response (3–10). Importantly, the role of TCTP in growth control is conserved in many eukaryotes. In *Arabidopsis*, loss of *TCTP* leads to growth retardation and reduced root growth (9). In *Drosophila*, knockdown of *Tctp* in imaginal discs decreases eye and wing sizes (7, 9). In addition, TCTP has been shown to be important for viability and proliferation of mammalian cells (7–9). Interestingly, previous studies have proposed that *Drosophila* Tctp functions as a guanine nucleotide exchange factor of *Drosophila* Ras homolog enriched in brain (dRheb) that controls the activity of the conserved growth regulator mechanistic target of rapamycin (mTOR) (7, 11). However, whether mammalian Tctp also regulates Rheb activity is unclear (12–14). Other studies have suggested that TCTP functions as a guanine nucleotide dissociation inhibitor of the translation elongation factor eEF1A, which can affect growth by affecting protein synthesis (15, 16). Given the importance of TCTP in growth control, crosstalk between TCTP and other signaling pathways is expected to be important for precise control of growth. Although crosstalk between TCTP and a few growth regulatory signaling pathways, including the Protein Kinase B (also known as AKT) signaling pathway and the Extracellular signaling-regulated kinase (ERK) signaling pathway, has been described in tissue culture cells (17), how such signaling crosstalk impinges on in vivo tissue growth control has not been characterized.

In animals, the Hippo (Hpo) signaling pathway controls organ growth by regulating cell proliferation and survival (18–22). In *Drosophila*, signaling from 2 protocadherins, Fat and Dachsous, is transduced via the core kinases, Hpo and Warts. Warts directly phosphorylates the *Drosophila* ortholog of Yes-associated protein 1 (Yap1), Yorkie (Yki), which is a transcription factor that activates genes controlling proliferation and survival. Furthermore, the *Drosophila* ortholog of Neurofibromatosis type 2, Merlin, and the *Drosophila* ortholog of kidney and brain expressed protein (KIBRA) form a protein complex that can function upstream of Hpo and Warts (23–25). Crosstalk between the Hippo pathway and the IGF/insulin pathway has been described in *Drosophila* and mammalian cells (26–28). Akt1 protein is upregulated in mosaic clones with decreased Hpo signaling or increased Yki expression (26). In addition, *Drosophila* midgut tumors induced by activation of Yki in intestinal stem cells (ISCs) cause an increase in Akt1 protein expression and phosphorylation, presumably because of a systematic increase in expression of multiple IGF/insulin signaling pathway components, including *Drosophila insulin-like peptide 3* (*dilp3*), *insulin-like receptor* (*InR*), and *Akt1*, in the midgut (29). Considering the conserved role of TCTP in growth control, interaction between the Hpo pathway and TCTP is likely. Nevertheless, such signaling crosstalk between TCTP and the Hpo signaling pathway has not been reported.

Stem cells play a critical role in tissue growth during normal homeostasis and tissue regeneration (30). In the adult *Drosophila* midgut epithelium, ISCs undergo cell division to generate themselves

Significance

Translationally controlled tumor protein (TCTP) is a conserved protein that regulates tissue growth in various multicellular organisms. Although precise control of growth is an integral part of the tissue repair process, it has remained unclear whether and how TCTP functions during tissue regeneration. By employing *Drosophila* genetics, we demonstrate that TCTP plays a crucial role in regeneration of damaged intestinal epithelium in part by regulating protein translation. Furthermore, we elucidate an unexpected crosstalk between TCTP and 2 key growth regulatory signaling pathways in animals. Altogether, our study provides insights into the mechanisms by which TCTP precisely controls tissue growth during animal development and regeneration.

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and 2 other cell types, enteroblasts (EBs) and preenteroendocrine cells (pre-EEs) (31–33). Subsequently, EBs and pre-EEs differentiate into enterocytes (ECs) and EEs, respectively (31–33). Thus, division of ISCs is critical for maintenance of midgut epithelium and regrowth of damaged midgut epithelium. Although TCTP is a conserved regulator of growth, the role of TCTP in stem cells is poorly understood. In this study, we demonstrate that TCTP is required for ISC proliferation and characterize its crosstalk with other growth regulatory signaling pathways, the AKT and Hpo signaling pathways, that control ISC proliferation during normal homeostasis and tissue regeneration.

Results

***Tctp* Is Expressed in the Midgut and Maintains Stem and Progenitor Cells.** In *Drosophila*, the roles of Tctp have been studied mainly in imaginal discs and other developing tissues. Although ISCs play crucial roles in controlling midgut size during normal homeostasis and tissue regeneration (30, 34–37), the function of Tctp in ISCs has not been addressed. To investigate its role in ISCs, we first tested whether Tctp was expressed in the adult midgut by staining with an antibody specifically recognizing *Drosophila* Tctp (7). Previously, it was shown that Tctp was ubiquitously expressed in *Drosophila* wing imaginal discs (7). Similarly, Tctp is expressed throughout the midgut, and enriched in the cytoplasm of all cell types in the midgut (Fig. 1*A*). Knockdown of *Tctp* by expressing a dsRNA targeting *Tctp* (7) with *esg-GAL4*, *UAS-GFP*, *tub-GAL80^{t}*, (referred to as *esg^{ts}* hereafter; *esg^{ts}>UAS-Tctpi*) reduced Tctp specifically in ISCs and EBs, while overexpression of *Tctp* (*esg^{ts}>UAS-Tctp*) significantly increased Tctp level (*SI Appendix*, Fig. S1 *A* and *B*). Interestingly, *Tctp* knockdown with *esg^{ts}* resulted in a reduction in ISCs and EBs, while *Tctp* overexpression had the opposite effects (Fig. 1 *B* and *C*). Furthermore, Tctp is important for maintaining ISCs, since knockdown or overexpression of *Tctp* decreased or increased the number of cells expressing Delta (Dl), a marker of ISCs (Fig. 1 *B* and *D*). To confirm these observations, we used a *Dl-LacZ* line, an enhancer trap line that expresses *LacZ* under the control of the *Dl* promotor. Knockdown of *Tctp* using *UAS-Tctpi* significantly reduced the cells expressing LacZ (*SI Appendix*, Fig. S1*C*). Conversely, overexpression of *Tctp* increased the number of cells expressing LacZ (*SI Appendix*, Fig. S1*C*). Notably, inhibition of apoptosis by expressing p35 failed to rescue the reduction in ISCs caused by *Tctp* knockdown (*SI Appendix*, Fig. S2), indicating that an increase in apoptosis might not account for the phenotype caused by *Tctp* knockdown. Altogether, these results suggest that Tctp plays an important role in maintenance and growth of ISCs during normal homeostasis in the adult *Drosophila* midgut.

***Tctp* Is Required for Tissue Damage-Induced ISC Proliferation.** Feeding pathogenic bacteria or administration of bleomycin or dextran sulfate sodium (DSS) cause damage to the midgut epithelium, which leads to proliferation of ISCs to replenish the cells lost during tissue damage (30). Indeed, feeding bleomycin significantly increased ISCs and EBs, which were marked with GFP expressed by *esg^{ts}* (Fig. 2*A*). Importantly, Tctp protein levels were significantly increased in ISCs and EBs after feeding bleomycin (Fig. 2 *A* and *B*), raising the possibility that Tctp might play a role in regeneration of the midgut epithelium in *Drosophila*. Thus, we examined whether manipulation of Tctp levels could alter proliferation of ISCs upon tissue damage. We found that knockdown of *Tctp* significantly reduced ISC division during normal homeostasis (Fig. 2 *C*–*F*). In contrast, ectopic expression of *Tctp* had little effect on homeostatic ISC division, since we found that the number of cells stained with anti-phospho-histone H3 antibody were not significantly altered by overexpression of *Tctp* with *esg^{ts}* (Fig. 2 *D* and *F*). These results suggest that a decrease in cell division contributes to the reduction in ISCs and EBs by knockdown of *Tctp* (Fig. 1 *C* and *D*). Feeding bleomycin significantly increased the

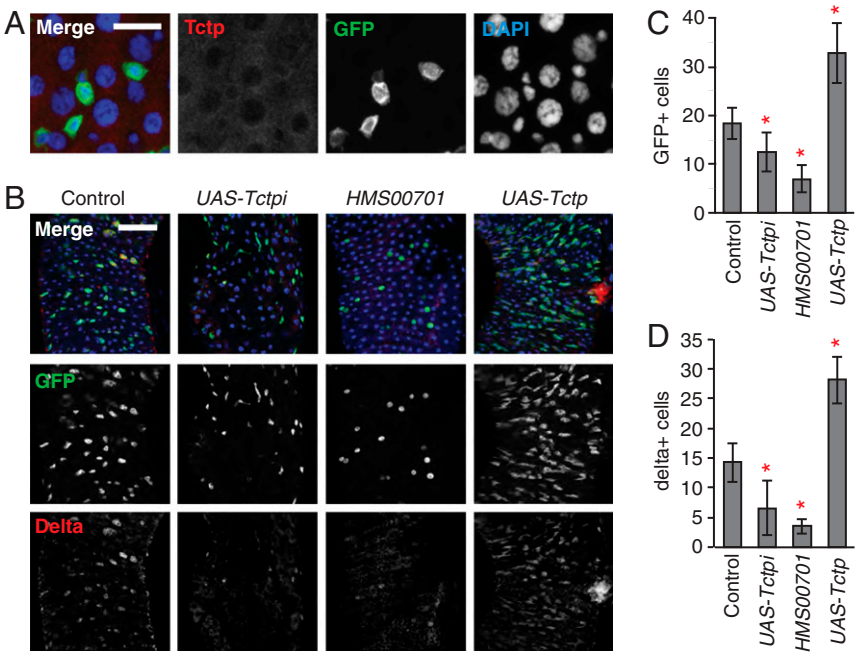

Fig. 1. Tctp is required for maintaining ISCs during tissue homeostasis. (*A*) Tctp staining in the adult *Drosophila* midgut. ISCs and EBs were marked with *esg^{ts}* (*esg-GAL4*, *UAS-GFP*, *Tub-GAL80^{ts}*) by incubating flies at 29 °C for 5 d. (Scale bar: 20 μm.) (*B*–*D*) *Tctp* knockdown reduces intestinal stem cell number. (*B*) Midgut images. Transgenes and GFP are induced by *esg^{ts}*. Two Tctp RNAi lines, *UAS-Tctpi* and *HMS00701*, were used. ISCs are marked by staining Dl. (Scale bar: 50 μm.) (*C*) Number of GFP-expressing cells in midguts. Number of ISCs and EBs in an area of interest (300 × 300 pixel^2) were counted. (*D*) Number of ISCs in midguts. Number of Dl-expressing cells in an area of interest (300 × 300 pixel^2) were counted. Mean ± SDs are shown. *$P < 0.05$ (Student *t* test) compared with control.

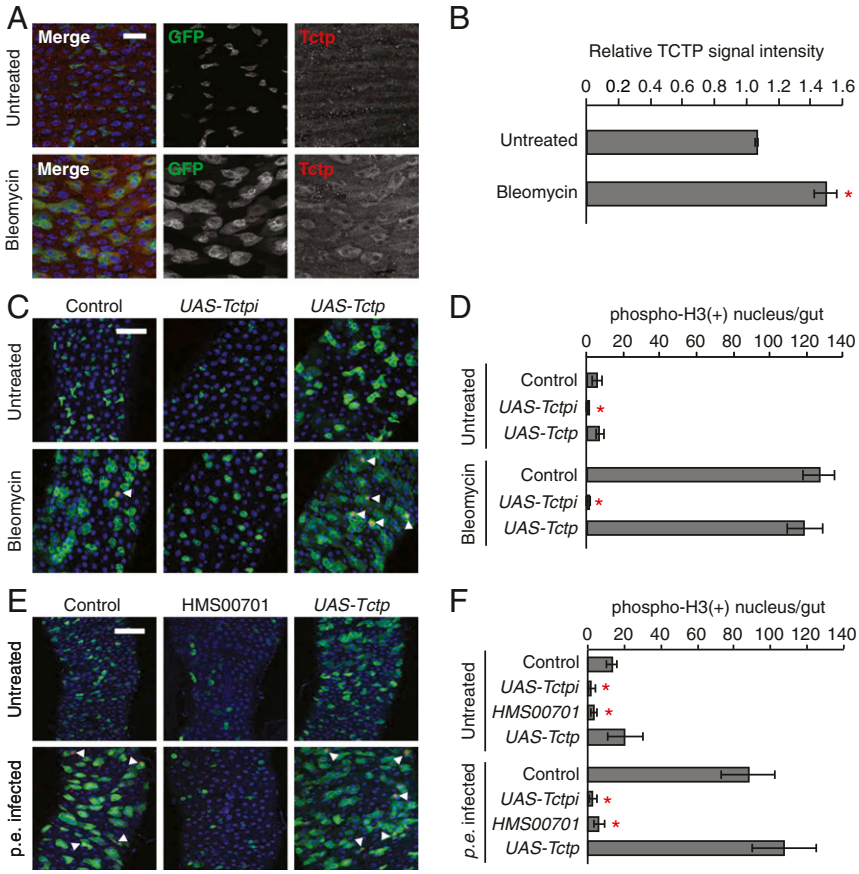

Fig. 2. Tctp is required for ISC proliferation during tissue homeostasis and damage. (*A*) Tctp staining in normal and damaged midguts. $egs^{ts}/^{+}$ flies were fed on normal food and bleomycin-treated food at 29 °C. Midguts were dissected, and Tctp levels were compared by immunostaining. Maximum projections of 4 to 5 stacks covering an epithelial layer are presented. (Scale bar: 20 μm.) (*B*) Relative Tctp protein levels. Similar levels of background Tctp signal were detected in every cell type in control midguts. Therefore, Tctp signal intensity in GFP+ cells were measured and then normalized to Tctp signal intensity in nearby ECs. We acquired 10 measurements from a posterior midgut and then calculated an average. Three midgut images for each condition were used for quantification. Mean ± SDs are shown. $*P < 0.05$ (Student *t* test) compared with untreated. (*C*) Midgut images with or without bleomycin feeding. GFP marks ISCs and EBs. *UAS-Tctpi* (7) is a RNAi line targeting *Tctp*. (Scale bar: 50 μm.) (*D*) Quantification of phospho-histone H3 signals in midgut with or without bleomycin feeding. Mean ± SDs are shown. $*P < 0.05$ (Student *t* test) compared with control. (*E*) Midgut images with or without *P. entomophila* feeding. HMS00701 is a short hairpin RNAi line targeting *Tctp*. (Scale bar: 50 μm.) (*F*) Quantification of phospho-histone H3 signals in midgut. Mean ± SDs are shown. $*P < 0.05$ (Student *t* test) compared with control.

number of dividing cells in control midguts (Fig. 2 *C* and *D*). In contrast, ISCs with reduced amounts of Tctp failed to proliferate in response to tissue damage induced by feeding bleomycin (Fig. 2 *C* and *D*). Furthermore, ingestion of pathogenic bacteria, *Pseudomonas entomophila*, significantly increased ISC division in control individuals and the midguts with *Tctp* overexpression, while knockdown of *Tctp* almost abolished ISC proliferation induced by *P. entomophila* ingestion (Fig. 2 *E* and *F*). Altogether, these results show that Tctp plays a crucial role in ISC proliferation during normal homeostasis and tissue damage.

The Hippo Signaling Pathway Controls the Abundance of Tctp in ISCs. In *Drosophila*, intestinal tissue regeneration involves multiple signaling pathways, including JAK/STAT, insulin/IGF, EGFR/Ras/Mapk, and Hippo signaling pathways (30, 37–42). To understand how Tctp protein abundance is controlled in ISCs and EBs during tissue regeneration, we examined whether activation of the signaling pathways involved in tissue regeneration alter Tctp protein levels in ISCs and EBs. Activation of the *insulin receptor* gene (*InR*) increases Akt1 signaling as well as mTOR signaling. In human HeLa and HT29 colon cancer cells, Akt and mTOR have been shown to be involved in the regulation of TCTP protein abundance without affecting *TCTP* mRNA levels (43). Expression of myristoylated-Akt1 with esg^{ts} caused a marginal increase in Tctp protein levels, while expression of an active form of the *Insulin-like receptor*, InR^{act}, failed to increase Tctp proteins in ISCs and EBs (*SI Appendix*, Fig. S3*A*). Similarly, activation of mTOR, by overexpression of Rheb, did not alter Tctp protein abundance (*SI Appendix*, Fig. S3*B*). In addition, neither expression of an oncogenic *Ras* allele, Ras^{V12}, nor activation of JAK/STAT signaling by expression of a constitutively active form of *hopscotch*, hop^{Tum}, significantly altered Tctp protein abundance in ISCs and EBs (*SI Appendix*, Fig. S3 *C* and *D*). Strikingly, however, manipulation of Hpo signaling in ISCs and EBs greatly altered Tctp protein levels. Either expression of an active *yki* allele ($yki^{3S/A}$) or knockdown of the upstream kinase *hpo* dramatically increased Tctp protein levels (Fig. 3*A*). The Hpo signaling pathway controls organ growth and tumor formation in many animals (18, 19, 21, 44–47). The human ortholog of *yki*, *yap1* is an oncogene that is amplified and/or activated in many types of cancers (47). Since we observed that Tctp protein levels were elevated by activation of Yki, we tested whether *Tctp* was required for cell overproliferation induced by Yki activation in the *Drosophila* midgut. Knockdown of *Tctp* completely suppressed cell proliferation induced by expression of $yki^{3S/A}$ (Fig. 3*B*), indicating that Tctp is essential for Yki-induced cell overproliferation. Notably, we found that *Tctp* mRNA levels were

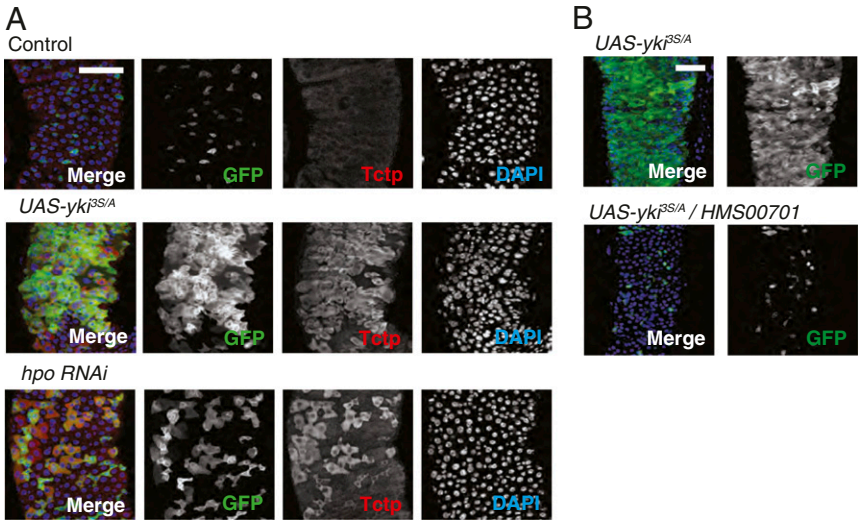

Fig. 3. Yorkie activation increases Tctp protein levels in ISCs. (*A*) Tctp staining in midguts. Transgenes were induced for 3 d with *esg*^{ts} by shifting to 29 °C. Tctp was stained with anti-Tctp antibody (7). (*B*) Midgut images. Transgenes were expressed for 6 d using *esg*^{ts} by shifting to 29 °C.

unaltered by activation of Yki signaling (*SI Appendix*, Fig. S4), suggesting that the regulation of Tctp protein abundance by Yki involves a posttranscriptional mechanism. Interestingly, previous studies have shown that TCTP protein expression was regulated posttranscriptionally (1, 2). Serum stimulation rapidly increased *TCTP* mRNA translation and protein abundance (1). Altogether, our findings indicate that the Hpo signaling pathway plays a critical role in controlling the abundance of Tctp protein in the *Drosophila* midgut.

Tctp Overexpression Does Not Induce Phosphorylation of 4E-BP in the Intestine. A previous study suggested that Tctp modulates mTOR activity by functioning as a guanine nucleotide exchange factor for Rheb in *Drosophila* wing imaginal discs (7). If Rheb is a direct effector of Tctp, Tctp overexpression may recapitulate the phenotypes caused by Rheb overexpression. Rheb overexpression in ISCs and EBs caused a significant increase in cell size, while cell numbers were not altered (Fig. 4), which is consistent with the proposed function of *Drosophila* mTOR in regulation of cell growth (48–50). In contrast, overexpression of *Tctp* caused an increase in cell number and a marginal increase in cell size (Fig. 4). mTOR directly phosphorylates 4E-BP, a negative regulator of translation. Overexpression of Rheb increased phosphorylation of 4E-BP in ISCs and EBs, while Tctp overexpression failed to increase phosphorylation of 4E-BP (Fig. 4). These results suggest that Rheb may not be the direct effector of Tctp in ISCs and EBs in the *Drosophila* midgut.

Tctp Forms a Complex with Translation Elongation Factors and Genetically Interacts with Genes Involved in Translation. Previous studies have shown that human TCTP forms a complex with eukaryotic elongation factors and inhibits guanine nucleotide dissociation from eEF1A (15, 51). To elucidate potential interactions between Tctp and the translational machinery, we systematically identified Tctp-interacting proteins by affinity purification coupled with mass spectrometry (*SI Appendix*, Table S1) (52). We found that Ef1alpha48D, Ef1gamma, and Ef1beta are enriched in Tctp pull-down samples compared with control pull-down samples; the peptides corresponding to the elongation factors were detected to be 3 to 378 times more enriched in Tctp pull-down samples (Fig. 5*A*). Systematic analysis of the Tctp-interactome uncovered a number of proteins in ribosomes and translation elongation complexes (Fig. 5*B*), suggesting that Tctp might be involved in regulation of translation. To examine the general impact of Tctp on translation, we analyzed the size of the nucleolus as it is involved in ribosome biogenesis. ECs are generally larger than ISCs and EBs; thus, their nucleoli also appear bigger than the nucleoli of ISCs and EBs. We selectively manipulated ISCs and EBs by overexpressing Tctp with *esg*^{ts}. Notably, Tctp overexpression with *esg*^{ts} caused an increase in the nucleolus size in ISCs and EBs (Fig. 5*C*, arrows), while the size of the nucleoli of ECs remained unaltered. Furthermore, Tctp overexpression significantly increased incorporation of L-homopropagylrglycine (HPG) (Fig. 5*D*), indicating that Tctp overexpression increased general translation in ISCs and EBs. As Tctp protein levels were

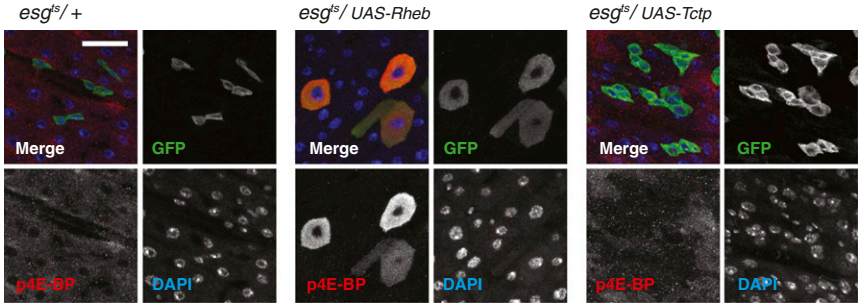

Fig. 4. Tctp overexpression does not recapitulate the phenotypes caused by Rheb overexpression. Transgenes were induced with *esg*^{ts} for 5 d at 29 °C. Midguts were dissected and stained for phospho-4E-BP. (Scale bar: 25 μm.)

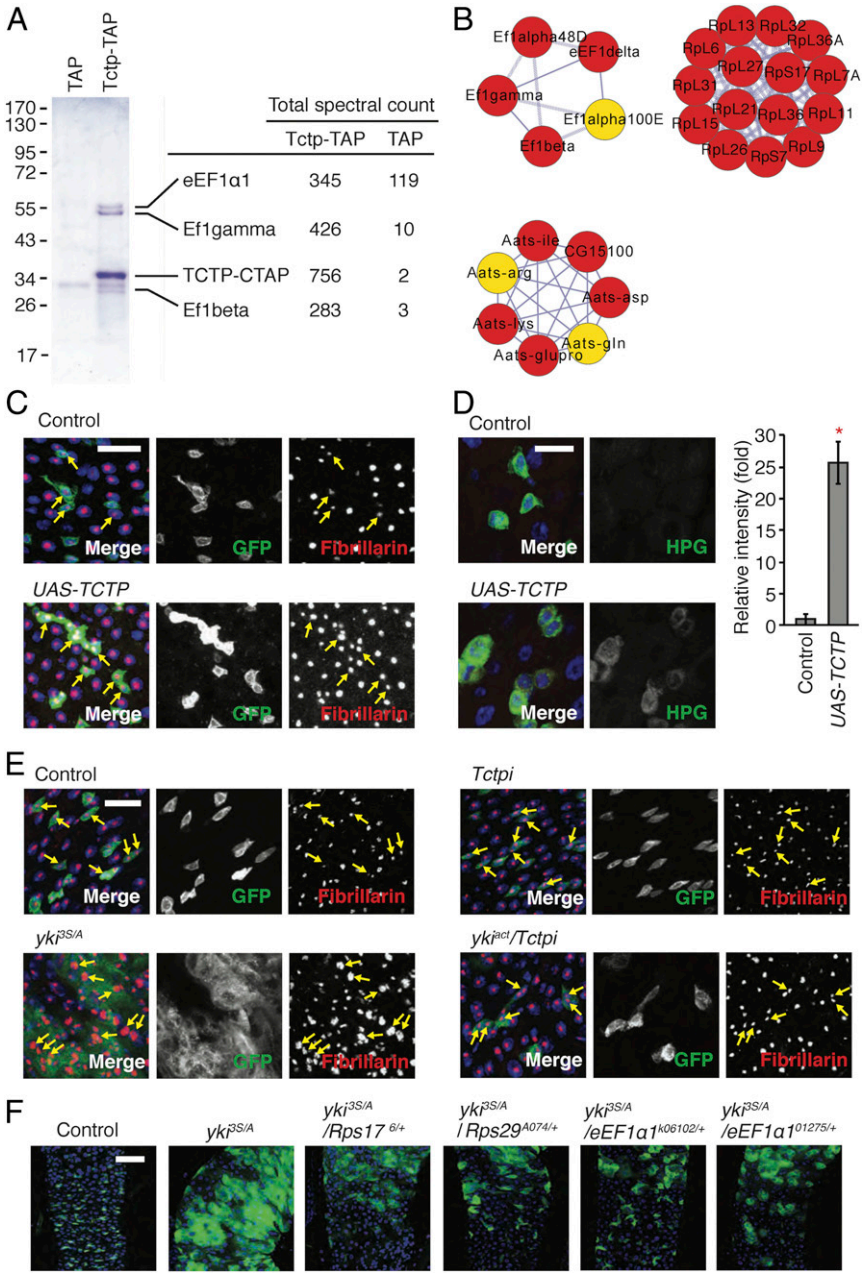

Fig. 5. Tctp interacts with the translation machinery. (*A*) Coomassie brilliant blue stain. TAP alone or Tctp-TAP was purified from S2R^{+} cells, and then purified protein complexes were separated by SDS/PAGE. We annotated the proteins based on molecular size and spectral count abundance in the samples measured by mass spectrometry. (*B*) Protein subcomplexes present in Tctp MS result. Red nodes are proteins enriched more than 2-fold in Tctp-TAP pull-down compared with TAP alone pull-down (Dataset S1). Yellow nodes are proteins predicted to be part of the protein complexes, but not enriched in Tctp-TAP pull-down. (*C*) Fibrillarin staining of nucleolus. Midguts were dissected and stained with anti-Fibrillarin antibody. *UAS-Tctp* was expressed for 6 d, using *esg*^{ts}, by shifting to 29 °C. (Scale bar: 25 μm.) (*D*) Assessment of protein synthesis. Incorporation of Homopropargylglycine (HPG) in midguts was measured ex vivo. Transgenes were induced for 2 d before dissection. (Scale bar: 10 μm.) (*E*) Fibrillarin staining. Midguts were stained with anti-Fibrillarin antibody. *UAS-yki*$^{3S/A}$ and *UAS-Tctpi* were expressed for 4 d, using *esg*^{ts}, by shifting to 29 °C. (Scale bar: 20 μm.) (*F*) Genetic interaction. Removing 1 copy of *Rps17* (BDSC#6177), *Rps29* (BDSC#16031), *EF1alpha48D* (BDSC#10397), or *Ef1alpha48D* (BDSC#11037) significantly suppressed Yki$^{3S/A}$-induced cell proliferation. (Scale bar: 50 μm.)

increased by activation of Yki, we examined nucleolus size in Yki-induced cell overproliferation in midguts. Similarly, nucleolus size was greatly increased by expression of *yki*$^{3S/A}$ with *esg*^{ts} (Fig. 5*E*), indicating that ribosome biogenesis is increased during the overproliferation induced by activation of Yki in ISCs and EBs. Notably, we found that this increase in nucleolus size induced by Yki activation could be reversed by *Tctp* knockdown (Fig. 5*E*). These results suggest that an increase in Tctp protein on activation of Yki may contribute to the increase in nucleolus size while we speculate that additional factors, such as dilp3 and Myc (29, 53–55), are involved in nucleolus size control on Yki activation. Of importance, removal of 1 copy of genes involved in translation, such as *Ef1alpha48D*, *Rps17*, and *Rps29*, was sufficient to significantly reduce the cell proliferation induced by activation of Yki (Fig. 5*F*), suggesting that translation control could be an important process for Yki-induced cell overproliferation. Altogether, our results demonstrate that Tctp forms a complex with translational machinery and contributes to

an increase in nucleolus size in vivo, which may influence translation in ISCs and EBs.

Tctp Impacts Akt1 Activity, Which Controls Growth. Observations in *Drosophila* and in human cells have proposed that TCTP affects a number of signaling pathways. Since we found that Tctp is essential for ISC proliferation induced by tissue damage and Yki activation, we tested whether Tctp could regulate signaling pathways involved in ISC proliferation. Interestingly, Tctp overexpression failed to significantly alter Hpo signaling and JAK/STAT signaling (*SI Appendix*, Fig. S5 *A* and *B*). Previously, it was shown that overexpression of TCTP increased AKT phosphorylation in MCF10A cells (56). Similarly, we found that overexpression of *Tctp* in ISCs and EBs increased phosphorylation of Akt1 in vivo (Fig. 6*A*). However, in contrast to the observations in human cells, ectopic expression of *Tctp* in ISCs and EBs also increased Akt1 protein levels (Fig. 6*B*).

Activation of Yki in ISCs and EBs increases Akt1 phosphorylation, which is possibly mediated by up-regulation of Dilp3 and multiple IGF/insulin signaling pathway components (29). Given the role of Tctp in Yki-induced cell proliferation (Fig. 3*B*), we examined whether Tctp contributed to the increase in Akt1 phosphorylation induced by activation of Yki with esg^{ts}. Occasionally, we noticed that Akt1 phosphorylation is also increased in adjacent cells that are not clearly labeled with GFP. This cell nonautonomous increase in Akt1 phosphorylation might be explained by an increase in Dilp3 expression in the tissue (29). Nevertheless, *Tctp* knockdown reduced Akt1 phosphorylation induced by activation of Yki in ISCs and EBs (Fig. 6*C*). Notably, elevation of Akt1 signaling by expressing myristolyated-Akt1 could rescue, at least partially, the cell proliferation defect caused by *Tctp* knockdown (Fig. 6*D*), suggesting that Akt1 is a critical mediator of Tctp signaling in vivo. Altogether, our results demonstrate that Tctp is a critical determinant of Akt1 activity in ISCs, and that elevation of Tctp during Yki activation contributes to an increase in Akt1 phosphorylation, which is known to promote cell growth.

Discussion

Multiple signaling pathways impinge on growth control, and understanding how these signaling pathways converge to achieve precise growth control is a question of fundamental interest. In *Drosophila*, *Tctp* loss results in a reduction in organ growth, which was proposed to be due to a decrease in cell growth. In *Arabidopsis*, *AtTCTP* loss of function decreases cell proliferation and survival, which leads to retardation in growth and reduction in organismal size. Strikingly, expression of *Drosophila Tctp* fully rescued the phenotypes caused by *AtTCTP* loss of function in *Arabidopsis*, suggesting that the role of TCTP in growth control is widely conserved (9). The role of the Hpo signaling pathway in growth control is also well conserved across species. In particular, Yki and its mammalian ortholog Yap1 control organ size and tumor growth. Here, we describe crosstalk between these 2 conserved regulators of growth: the Hpo signaling pathway and Tctp. We found that the Hpo signaling pathway is a critical determinant of Tctp protein levels in *Drosophila* ISCs and EBs. Activation of Yki or inhibition of Hpo leads to a significant increase in Tctp protein, which can potentially promote growth by controlling multiple growth regulatory processes. Importantly, Tctp is required for ISC proliferation upon tissue damage. *Tctp* knockdown

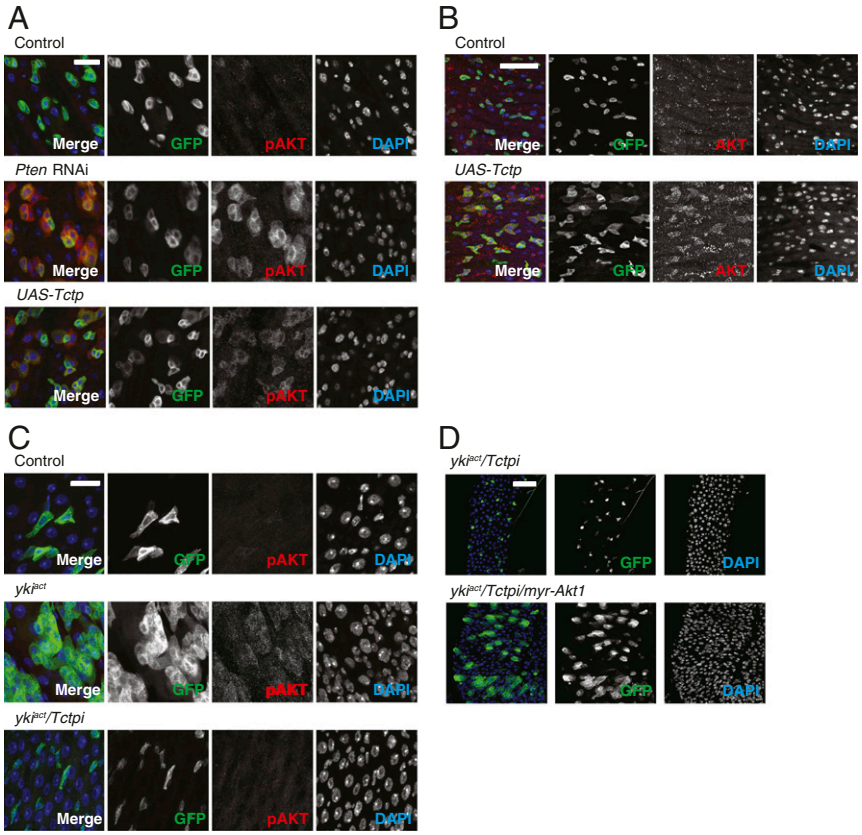

Fig. 6. Tctp controls Akt1 phosphorylation in vivo. (*A*) Detection of Akt1 phosphorylation. Transgenes were expressed for 6 d with esg^{ts}. *Pten RNAi* was used as a positive control. (Scale bar: 25 μm.) (*B*) Detection of Akt1. (Scale bar: 50 μm.) (*C*) Immunostaining for phospho-Akt1. Transgenes were induced for 4 d with esg^{ts}. Akt1 phosphorylation was detected by staining midguts with anti-phospho-Akt1 antibody. (Scale bar: 20 μm.) (*D*) Midgut images. Transgenes were induced for 5 d with esg^{ts}. (Scale bar: 50 μm.)

almost completely abolished ISC proliferation after tissue damage induced by feeding bleomycin, DSS, or pathogenic bacteria. Given the role of Yki in ISC proliferation during tissue damage, crosstalk between Hpo signaling and Tctp might play a critical role during tissue regeneration. Interestingly, our observations do not support the proposed role of Tctp as a Rheb guanine nucleotide exchange factor. Instead, our results suggest that Tctp impinges on 2 fundamental pathways that are important for growth control. Tctp forms a complex with multiple proteins involved in translation, and genetically interacts with ribosomal subunits, which can be at least partially explained by the proposed role of TCTP as a guanine nucleotide dissociation inhibitor of translation elongation factor eEF1A. Moreover, our study shows that Tctp increases both Akt1 protein abundance and phosphorylation in vivo. Nevertheless, the molecular mechanisms by which Tctp impinges on general translation and Akt1 activity require further investigation. Given the observation that Tctp forms a complex with a number of proteins involved in translation, it is interesting to speculate that an elevation in general translation mediated by Tctp could affect Akt1 phosphorylation by increasing the abundance of rate-limiting proteins in the insulin signaling pathway.

Altogether, our study unravels signaling crosstalk, comprising 3 well-characterized growth regulators, Yki, Tctp, and Akt1, that control ISC proliferation during tissue regeneration and Yki-induced cell overproliferation. Given the importance of the mammalian orthologs of Yki, Tctp and Akt1, in tumor growth, it will be of interest to examine whether the Yap1-TCTP-Akt signaling crosstalk plays a similar role in growth control of cancers, especially those with higher Yap1 activity.

Materials and Methods

Fly Stocks and Manipulation of Intestinal Stem Cells and Enteroblasts. *esg*^{ts} refers to *esg-GAL4*, *UAS-GFP*, *tub-GAL80*^{ts} (II) (57). *UAS-yki*^{act} (*w*;; UAS-yki.S111A.S168A.S250A.V5*, #28817) (58), *UAS-Rheb* (II) (BDSC#9688), and UAS-Rheb (III) (BDSC#9689) were obtained from the Bloomington *Drosophila* Stock Center (BDSC). RNAi lines from the Transgenic RNAi Project (TRiP; https://fgr.hms.harvard.edu/) were HMS00701 (BDSC#32911; *Tctp*), HMS00006 (BDSC#33614; *hpo*), and HMS00044 (BDSC#33643; *Pten*). In addition, we used *UAS-myr-Akt1* (III), *UAS-hop*^{tum} (II), *UAS-Ras*^{V12} (II), *UAS-rpr*, *UAS-p35*, and *UAS-InR*^{act}. *UAS-TCTP* (II) and *UAS-TCTPi* (II) are gifts from Dr. Choi (KAIST, South Korea). *RpS17*^{6}/TM2 (#6177), *RpS29*^{A074}/TM3 (#16031), *eEF1α1*^{k06102}/CyO (#10397), and *eEF1α1*^{01275}/CyO (#11034) were obtained from BDSC.

To induce transgenes in intestinal stem cells and enteroblasts, we followed the experimental procedures described previously (57). Briefly, crosses were set up with *esg*^{ts} at room temperature and, after 3 d of incubation at room temperature, transferred to 18 °C to restrict the expression of the Gal4-induced transgenes during development. Next, 0- to 4-d-old adult progenies were collected and placed at 29 °C to induce the transgenes. Progenies from a cross between *esg*^{ts} and *w*^{1118} were used as controls. During incubation at 29 °C, flies were transferred onto fresh food every 2 d.

Immunostaining and Antibodies. Before dissection, female flies were fed 4% sucrose for 3 to 6 h to remove food from the gut. Guts were dissected in PBS and fixed in PBS with 4% paraformaldehyde for 25 min. After washing 3 times with PBS, samples were blocked for 45 min in PBS with 0.2% Triton X-100 and 5% normal donkey serum. Gut tissues were incubated with primary antibody overnight at 4 °C in PBS with 0.2% Triton X-100 and 5% normal donkey serum and then washed 3 times with PBS. Alexa Fluor secondary antibodies (Molecular Probes) were diluted in PBS with 0.2% Triton X-100 and 5% normal donkey serum and treated for 1 h at room temperature. Tissue samples were mounted with Vectashield mounting medium (Vector Laboratories) and imaged with Leica SP2 confocal microscopy.

Antibodies used in this study are the following: rabbit anti-Tctp (gift from Dr. Choi at KAIST), rabbit anti-Akt (Cell Signaling), rabbit anti-phospho-*Drosophila* Akt (Cell Signaling), anti-phospho-Histone-H3 (Millipore), rabbit anti-β-Galactosidase (Cappel), rabbit anti-phospho-4E-BP (Cell Signaling), and chicken anti-Fibrillarin (EnCor Biotechnology).

Midgut Damage Experiments. To induce tissue damage, fly cornmeal/agar food was melted and mixed well with a final concentration of 25 μg/mL bleomycin (Calbiochem #203408) or 3% DSS (MB Biomedicals #160110). Flies were fed with bleomycin or DSS food for 2 d at 29 °C.

Pseudomonas entomophila from −80 °C stock was incubated in 100 mL LB medium in a 250-mL flask with 1: 1,000 Rifampicin (SIGMA) at 170 rpm at 30 °C for 40 h. Next, 100 mL culture was centrifuged at 3,000 rpm for 15 min. The supernatant was removed. The bacteria pallet was suspended with 5 mL 4% sucrose, and 500 μL *P. entomophila* solution was applied on a piece of filter paper (3.75 cm × 2.5cm) and put into empty vials. The flies that had been shifted to 29 °C for 7 d with normal food were transferred to these vials and kept at 29 °C for additional 20 h before dissection followed by immunostaining; 4% sucrose with no bacteria was used as control.

Affinity Purification and Tandem Mass Spectrometry. To generate the DNA constructs for tandem affinity purification, we subcloned the coding sequences of *GFP* or *Tctp* in the pMK33-CTAP vector (59). S2R^{+} cells were maintained in Schneider's Insect Medium (Invitrogen) supplemented with 10% heat-inactivated FBS at 25 °C. To generate stable cells, S2R^{+} cells were transfected with the pMK33-CTAP constructs, using Effectene transfection reagent (Qiagen). Stable cells were selected by exposing the transfected cells to 200 μM Hygromycin (Calbiochem, 40051).

Affinity purification and mass spectrometry experiments for Tctp or GFP control were performed in duplicates, as described previously (52). Peptides resuspended in Mass Spec buffer were analyzed on a LTQ Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific) equipped with an Agilent 1200 binary pump (Agilent Technologies). MS/MS spectra were matched to peptide sequences using SEQUEST (60) and a composite database containing the translated sequences of all predicted ORFs of *Drosophila melanogaster* and its reversed complement. Peptide spectral matches were filtered to 1% false discovery rate, using the target-decoy strategy (61) combined with linear discriminant analysis (62).

To analyze protein complexes enriched in the interactomes, we applied COMPLETE (63), a protein complex-based enrichment analysis tool. The enriched complexes were visualized using the Cytoscape network visualization software (https://cytoscape.org/).

Measuring Protein Synthesis. We employed a metabolic labeling approach based on incorporation of the noncanonical amino acid L-HPG (Life Technologies) into proteins, followed by chemoselective fluorescence, using the Click-iT cell reaction buffer kit (Life Technologies). To label newly synthesized proteins, we added 50 μM HPG in PBS to the freshly dissected guts for 30 min. Then, the guts were washed with PBS and then fixed in 4% paraformaldehyde for 15 min. After washing with 3% BSA in 0.2 mL PBS twice, the guts were permeabilized in 0.2% Triton X-100 in 0.2 mL PBS and then washed and blocked with 0.2 mL 3% BSA in PBS. Click chemistry was performed according to the manufacturer's protocol to join the amino acid alkyne with Alexa Fluor 594-azide. Midguts were mounted using Vectashield (Vector Laboratories, H-1000), and fluorescence micrographs were acquired with Leica SP8 confocal microscope with 40×/1.25 oil objective lens. The median fluorescence intensity per cell from HPG-containing individual cells was measured with NIH ImageJ software and normalized by the intensity of the background area. Data represent $n = 20$ individual measures from 6 independent guts.

Data Availability. All data are available within this manuscript and the associated *SI Appendix*.

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1. G. Thomas, G. Thomas, H. Luther, Transcriptional and translational control of cytoplasmic proteins after serum stimulation of quiescent Swiss 3T3 cells. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5712–5716 (1981).
2. R. Yenofsky, I. Bergmann, G. Brawerman, Messenger RNA species partially in a repressed state in mouse sarcoma ascites cells. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5876–5880 (1982).
3. F. Li, D. Zhang, K. Fujise, Characterization of fortilin, a novel antiapoptotic protein. *J. Biol. Chem.* **276**, 47542–47549 (2001).
4. H. S. Kang *et al.*, Molecular identification of IgE-dependent histamine-releasing factor as a B cell growth factor. *J. Immunol.* **166**, 6545–6554 (2001).
5. M. Tuynder *et al.*, Biological models and genes of tumor reversion: Cellular reprogramming through tpt1/TCTP and SIAH-1. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 14976–14981 (2002).
6. W. W. J. van de Sande *et al.*, Translationally controlled tumor protein from Madurella mycetomatis, a marker for tumorous mycetoma progression. *J. Immunol.* **177**, 1997–2005 (2006).

7. Y. C. Hsu, J. J. Chern, Y. Cai, M. Liu, K. W. Choi, Drosophila TCTP is essential for growth and proliferation through regulation of dRheb GTPase. *Nature* **445**, 785–788 (2007).
8. L. Susini *et al*., TCTP protects from apoptotic cell death by antagonizing bax function. *Cell Death Differ.* **15**, 1211–1220 (2008).
9. F. Brioudes, A. M. Thierry, P. Chambrier, B. Mollereau, M. Bendahmane, Translationally controlled tumor protein is a conserved mitotic growth integrator in animals and plants. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16384–16389 (2010).
10. S. T. Hong, K. W. Choi, Antagonistic roles of Drosophila Tctp and Brahma in chromatin remodelling and stabilizing repeated sequences. *Nat. Commun.* **7**, 12988 (2016).
11. T. P. Le, L. T. Vuong, A. R. Kim, Y. C. Hsu, K. W. Choi, 14-3-3 proteins regulate Tctp-Rheb interaction for organ growth in Drosophila. *Nat. Commun.* **7**, 11501 (2016).
12. H. Rehmann *et al*., Biochemical characterisation of TCTP questions its function as a guanine nucleotide exchange factor for Rheb. *FEBS Lett.* **582**, 3005–3010 (2008).
13. X. Wang *et al*., Re-evaluating the roles of proposed modulators of mammalian target of rapamycin complex 1 (mTORC1) signaling. *J. Biol. Chem.* **283**, 30482–30492 (2008).
14. X. Dong, B. Yang, Y. Li, C. Zhong, J. Ding, Molecular basis of the acceleration of the GDP-GTP exchange of human ras homolog enriched in brain by human translationally controlled tumor protein. *J. Biol. Chem.* **284**, 23754–23764 (2009).
15. C. Cans *et al*., Translationally controlled tumor protein acts as a guanine nucleotide dissociation inhibitor on the translation elongation factor eEF1A. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 13892–13897 (2003).
16. R. Amson, J. Z. Kubiak, M. Van Montagu, A. Telerman, Could TCTP contribute to Armin Braun's paradigm of tumor reversion in plants? *Cell Cycle* **10**, 1 (2011).
17. M. Kim, J. Jung, K. Lee, Roles of ERK, PI3 kinase, and PLC-gamma pathways induced by overexpression of translationally controlled tumor protein in HeLa cells. *Arch. Biochem. Biophys.* **485**, 82–87 (2009).
18. I. K. Hariharan, Organ size control: Lessons from Drosophila. *Dev. Cell* **34**, 255–265 (2015).
19. F. X. Yu, B. Zhao, K. L. Guan, Hippo pathway in organ size control, tissue homeostasis, and cancer. *Cell* **163**, 811–828 (2015).
20. F. Zanconato, M. Cordenonsi, S. Piccolo, YAP/TAZ at the roots of cancer. *Cancer Cell* **29**, 783–803 (2016).
21. B. K. Staley, K. D. Irvine, Hippo signaling in Drosophila: Recent advances and insights. *Dev. Dyn.* **241**, 3–15 (2012).
22. D. Pan, The hippo signaling pathway in development and cancer. *Dev. Cell* **19**, 491–505 (2010).
23. R. Baumgartner, I. Poernbacher, N. Buser, E. Hafen, H. Stocker, The WW domain protein Kibra acts upstream of Hippo in Drosophila. *Dev. Cell* **18**, 309–316 (2010).
24. A. Genevet, M. C. Wehr, R. Brain, B. J. Thompson, N. Tapon, Kibra is a regulator of the Salvador/Warts/Hippo signaling network. *Dev. Cell* **18**, 300–308 (2010).
25. J. Yu *et al*., Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and expanded. *Dev. Cell* **18**, 288–299 (2010).
26. X. Ye, Y. Deng, Z. C. Lai, Akt is negatively regulated by Hippo signaling for growth inhibition in Drosophila. *Dev. Biol.* **369**, 115–123 (2012).
27. K. Straßburger, M. Tiebe, F. Pinna, K. Breuhahn, A. A. Teleman, Insulin/IGF signaling drives cell proliferation in part via Yorkie/YAP. *Dev. Biol.* **367**, 187–196 (2012).
28. M. Xin *et al*., Regulation of insulin-like growth factor signaling by Yap governs cardiomyocyte proliferation and embryonic heart size. *Sci. Signal.* **4**, ra70 (2011).
29. Y. Kwon *et al*., Systemic organ wasting induced by localized expression of the secreted insulin/IGF antagonist ImpL2. *Dev. Cell* **33**, 36–46 (2015).
30. A. Amcheslavsky, J. Jiang, Y. T. Ip, Tissue damage-induced intestinal stem cell division in Drosophila. *Cell Stem Cell* **4**, 49–61 (2009).
31. B. Ohlstein, A. Spradling, The adult Drosophila posterior midgut is maintained by pluripotent stem cells. *Nature* **439**, 470–474 (2006).
32. C. A. Micchelli, N. Perrimon, Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* **439**, 475–479 (2006).
33. X. Zeng, S. X. Hou, Enteroendocrine cells are generated from stem cells through a distinct progenitor in the adult Drosophila posterior midgut. *Development* **142**, 644–653 (2015).
34. H. Jiang, B. A. Edgar, Intestinal stem cells in the adult Drosophila midgut. *Exp. Cell Res.* **317**, 2780–2788 (2011).
35. H. Jiang, B. A. Edgar, Intestinal stem cell function in Drosophila and mice. *Curr. Opin. Genet. Dev.* **22**, 354–360 (2012).
36. L. E. O'Brien, S. S. Soliman, X. Li, D. Bilder, Altered modes of stem cell division drive adaptive intestinal growth. *Cell* **147**, 603–614 (2011).
37. H. Jiang, M. O. Grenley, M. J. Bravo, R. Z. Blumhagen, B. A. Edgar, EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in Drosophila. *Cell Stem Cell* **8**, 84–95 (2011).
38. H. Jiang *et al*., Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. *Cell* **137**, 1343–1355 (2009).
39. R. L. Shaw *et al*., The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. *Development* **137**, 4147–4158 (2010).
40. F. Ren *et al*., Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21064–21069 (2010).
41. P. Karpowicz, J. Perez, N. Perrimon, The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. *Development* **137**, 4135–4145 (2010).
42. B. K. Staley, K. D. Irvine, Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. *Curr. Biol.* **20**, 1580–1587 (2010).
43. U. A. Bommer *et al*., Growth-factor dependent expression of the translationally controlled tumour protein TCTP is regulated through the PI3-K/Akt/mTORC1 signalling pathway. *Cell. Signal.* **27**, 1557–1568 (2015).
44. F. X. Yu, K. L. Guan, The Hippo pathway: Regulators and regulations. *Genes Dev.* **27**, 355–371 (2013).
45. K. F. Harvey, X. Zhang, D. M. Thomas, The Hippo pathway and human cancer. *Nat. Rev. Cancer* **13**, 246–257 (2013).
46. X. Yang, T. Xu, Molecular mechanism of size control in development and human diseases. *Cell Res.* **21**, 715–729 (2011).
47. R. Johnson, G. Halder, The two faces of Hippo: Targeting the Hippo pathway for regenerative medicine and cancer treatment. *Nat. Rev. Drug Discov.* **13**, 63–79 (2014).
48. Y. Zhang *et al*., Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. *Nat. Cell Biol.* **5**, 578–581 (2003).
49. H. Stocker *et al*., Rheb is an essential regulator of S6K in controlling cell growth in Drosophila. *Nat. Cell Biol.* **5**, 559–565 (2003).
50. L. J. Saucedo *et al*., Rheb promotes cell growth as a component of the insulin/TOR signalling network. *Nat. Cell Biol.* **5**, 566–571 (2003).
51. H. Wu *et al*., Evolutionarily conserved binding of translationally controlled tumor protein to eukaryotic elongation factor 1B. *J. Biol. Chem.* **290**, 8694–8710 (2015).
52. Y. Kwon *et al*., The Hippo signaling pathway interactome. *Science* **342**, 737–740 (2013).
53. R. M. Neto-Silva, S. de Beco, L. A. Johnston, Evidence for a growth-stabilizing regulatory feedback mechanism between Myc and Yorkie, the Drosophila homolog of Yap. *Dev. Cell* **19**, 507–520 (2010).
54. M. Ziosi *et al*., dMyc functions downstream of Yorkie to promote the supercompetitive behavior of hippo pathway mutant cells. *PLoS Genet.* **6**, e1001140 (2010).
55. S. S. Grewal, L. Li, A. Orian, R. N. Eisenman, B. A. Edgar, Myc-dependent regulation of ribosomal RNA synthesis during Drosophila development. *Nat. Cell Biol.* **7**, 295–302 (2005).
56. J. Jung *et al*., Translationally controlled tumor protein induces human breast epithelial cell transformation through the activation of Src. *Oncogene* **30**, 2264–2274 (2011).
57. Y. Apidianakis, C. Pitsouli, N. Perrimon, L. Rahme, Synergy between bacterial infection and genetic predisposition in intestinal dysplasia. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 20883–20888 (2009).
58. H. Oh, K. D. Irvine, In vivo analysis of Yorkie phosphorylation sites. *Oncogene* **28**, 1916–1927 (2009).
59. P. Kyriakakis, M. Tipping, L. Abed, A. Veraksa, Tandem affinity purification in Drosophila: The advantages of the GS-TAP system. *Fly (Austin)* **2**, 229–235 (2008).
60. J. K. Eng, A. L. McCormack, J. R. Yates, An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* **5**, 976–989 (1994).
61. J. E. Elias, S. P. Gygi, Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nat. Methods* **4**, 207–214 (2007).
62. E. L. Huttlin *et al*., A tissue-specific atlas of mouse protein phosphorylation and expression. *Cell* **143**, 1174–1189 (2010).
63. A. Vinayagam *et al*., Protein complex-based analysis framework for high-throughput data sets. *Sci. Signal.* **6**, rs5 (2013).