Drosophila Casein Kinase 2 (CK2) Promotes Warts Protein to Suppress Yorkie Protein Activity for Growth Control*

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Background: Hippo signaling regulates Wts activity to phosphorylate and inhibit Yki and hence control tissue growth.

Results: CK2 enhances Wts-induced Yki phosphorylation to down-regulate Yki activity whereas it contributes to a role in cell survival.

Conclusion: Drosophila CK2 promotes Wts to suppress Yki activity.

Significance: We found that CK2, as a cell death inhibitor, may be a potential growth inhibitor as well.

Drosophila Hippo signaling regulates Wts activity to phosphorylate and inhibit Yki in order to control tissue growth. CK2 is widely expressed and involved in a variety of signaling pathways. In this study we report that Drosophila CK2 promotes Wts activity to phosphorylate and inhibit Yki activity, which is independent of Hpo-induced Wts promotion. In vivo, CK2 overexpression suppresses hpo mutant-induced expanded (Ex) up-regulation and overgrowth phenotype, whereas it cannot affect wts mutant. Consistent with this, knockdown of CK2 up-regulates Hpo pathway target expression. We also found that Drosophila CK2 is essential for tissue growth as a cell death inhibitor as knockdown of CK2 in the developing disc induces severe growth defects as well as caspase3 signals. Taken together, our results uncover a dual role of CK2; although its major role is promoting cell survival, it may potentially be a growth inhibitor as well.

The Hippo (Hpo)3 signaling pathway is one of the pathways that controls organ size during animal development. It regulates tissue growth by balancing cell proliferation and apoptosis and has been implicated in stem cell maintenance and tissue regeneration and repair (1–3). Its malfunction is implied to relate to a wide range of human cancers and diseases (4). Core to the Hpo pathway is a kinase cascade that leads to inhibited Yki activity by protein-protein interaction. These components interact with Yki through their PPX/Y motifs and Yki WW domains to restrict Yki in cytoplasm (28–30). These components include X-ray repair cross-complementing group 1 (xrc1), the Hippo (Hpo), Yorkie (Yki), WW domains to restrict Yki in cytoplasm (28–30). These components interact with Yki through their PPX/Y motifs and Yki WW domains to restrict Yki in cytoplasm (28–30). These components include X-ray repair cross-complementing group 1 (xrc1), the Hippo (Hpo), Yorkie (Yki), transcription factors, primarily Scalloped (31–33) and others including Homothorax (34), Teashirt (34), and Mad (35), in the nucleus to promote proliferation and inhibit apoptosis by inducing the expression of target genes, such as diap1, expanded, and bantam.

Casein kinase 2 (CK2) is a highly conserved serine/threonine protein kinase and a stable tetrameric complex consisting of two catalytic (α) subunits and two regulatory (β) subunits. The core of the CK2 tetramer is the dimer of the CK2 catalytic subunit CK2α that is a prerequisite for the formation of tetrameric CK2 complexes with the catalytic subunit CK2α (36, 37). CK2α enhances the stability of CK2 and modulates substrate specificity and activity of CK2α (38, 39). CK2 is constitutively active and ubiquitously expressed in a wide variety of cellular processes. It is a key suppressor of apoptosis and is essential for cell survival (40). An increase in its protein expression was detected in multiple forms of cancers (40–42). It has been shown that CK2 phosphorylates components in a variety of signaling pathways including Wnt, NF-κB, and Hedgehog pathways (43, 44). However, it is unknown whether CK2 is involved in Hpo signaling.


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In this study we found that CK2 overexpression promotes Wts to phosphorylate Yki and in this way to suppress Yki activity in vitro. The function of CK2 on Wts is independent of the Hpo-induced Wts promotion, as knockdown of Hpo cannot block this function. In vivo, we found that CK2 overexpression suppresses hpo mutant-induced expanded (Ex) up-regulation and overgrowth phenotype, although it cannot affect wts mutant at all. Consistently, up-regulation of Hpo pathway target gene expressions is observed in tissues with CK2 depletion, indicating that loss of CK2 increases Yki activity. Meanwhile, our in vivo evidence also suggests a necessity of Drosophila CK2 for normal tissue growth as it acts as a cell death inhibitor and its depletion in developing discs induces caspase3 severely. Taken together, our results uncover a dual role of CK2 on regulating tissue growth.

EXPERIMENTAL PROCEDURES

Cell Culture, Transfection, Immunoprecipitation, and Western Blotting—S2 cell were cultured in Drosophila Schneider’s Medium (Invitrogen) with 10% fetal bovine serum, 100 units/ml of penicillin, and 100 mg/ml streptomycin. Plasmid transfection was carried out using Lipofectamine (Invitrogen) according to the manufacturer’s instructions. A construct of ubiquitin-Gal4 was cotransfected with pUAST expression vectors for all transfection experiments. For immunoprecipitation, Drosophila S2 cells were cultured for 3 days and then were collected and lysed in immunoprecipitation buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1% Nonidet P-40, 10% glycerol, 1.5 mM EDTA, 10 mM NaF, 1 mM Na3VO4) with a protease inhibitor mixture (Sigma). Cell lysates were preclarified by Protein A beads (GE Healthcare) and then incubated with indicated antibodies. Western blot analysis were performed according to standard protocols as previously described. Antibodies used were mouse anti-Myc (1:5000, Sigma), mouse anti-FLAG (1:5000, Sigma), mouse anti-V5 (1:5000, Invitrogen), mouse anti-HA (1:1000, Developmental Studies Hybridoma Bank), mouse anti-histone (1:1000, Sigma), rabbit anti-Yki phosphorylated Ser-168 (Ser(P)-168) (1:500) was generated by Abgent.

RNA Interference in Drosophila S2 Cells—Double strand RNA (dsRNA) was designed and synthesized according to standard protocol. To perform knockdown experiment, S2 cells were diluted into 1 × 10⁶ cells/ml with serum-free medium for 1 h of starvation with 15 μg/ml indicated dsRNA.

Real-time PCR—Total RNA was extracted from S2 cells that express the indicated constructs using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. The resulting RNA was used to synthesize cDNA by ReverTra Ace synthesis kit (Toyobo). The real-time PCR was performed using ABI7500 System with SYBR Green real-time PCR Master Mix (Toyobo) reagent. Primer sequences are available upon request. Real-time PCR was repeated for three independent biological replicates. Rp49 was used as a normalization control for all of the PCR reaction.

In Vitro Kinase Assay—For in vitro kinase assay, bacterial purified GST-Yki proteins were incubated with immunoprecipitated Wts or CK2 (expressed in S2 cells) and ATP (0.5 mm) in a kinase reaction buffer (250 mM HEPES, pH 7.4, 0.2 mM EDTA, 1% glycerol, 150 mM NaCl, and 10 mM MgCl2). Proteins were then subjected for Western analysis with Yki Ser(P)-168 antibody.

Phosphorylation Mobility Shift Assay—For some phosphorylation mobility shift assays, Phos-Tag AAL-107 (FMS Laboratory, Hiroshima University, Japan) was introduced to enlarge the mobility shift. The operating procedure was according to the manufacturer’s instructions. For all the mobility shift assays, the protein samples ran in an SDS-PAGE gel under a low voltage.

Drosophila Strains—To construct CK2α and CK2β transgenes, larva cDNA fragments corresponding to Drosophila CK2α and CK2β coding sequence were amplified by PCR and cloned into a pUAST-FLAG vector. The generation of transgenes at attP locus have been described previously. CK2α RNAi (17520R1, 17520R2) and CK2β RNAi (15224R1, 15224R2) were ordered from the National Institute of Genetics (NIG). A CK2 point mutation (K66M) was generated by PCR-based site-directed mutagenesis and verified by DNA sequencing, and all of these cDNA fragments were cloned into the pUAST vector. Other stocks used in this study were described previously including wts¹⁷⁵2⁰x¹, hpo⁵⁰⁴⁰³, diap1-GFP₄₃, ex-lacZ, bantam sensor, GMR-Gal4, eyeless-Gal4, MS1096-Gal4, hh-Gal4, act>CD2>Gal4(27, 31). All flies and crosses were kept at 25 °C unless special indication.

Immunofluorescence Staining—Immunostaining of imaginal discs was carried out as described. Primary antibodies used in this studies include: rat anti-Ci (1:500) (31), mouse anti-CD2 (1:1000, AbD Serotec), mouse anti-Diap1 (1:50, a gift from Bruce A. Hay, California Institute of Technology), rabbit anti-Ex (1:50, a gift from Allen Lauhgon, University of Wisconsin), mouse anti-Myc (1:5000), mouse anti-FLAG (1:5000), rabbit anti-lacZ (1:1000) (31).

Microscopy and Data Analysis—Fluorescent microscopy was performed on a Leica LAS SP5 confocal microscope; confocal images were obtained using the Leica AF Lite system. Images were processed in Photoshop CS.

RESULTS

CK2 Promotes Yki Phosphorylation and Cytoplasmic Localization—CK2 is widely expressed and involved in a lot of cellular processes. Both CK2 and Hippo signaling have been demonstrated to control cell proliferation and apoptosis. We wondered if CK2 affects Hippo pathway activity. The phosphorylation status of Yki, especially of Yki Ser-168, reflects the activity of Yki (30). We detected a dramatic increase of Yki Ser(P)-168 upon CK2 coexpression (CK2α plus CK2β; Fig. 1A). Furthermore, a phosphorylation mobility shift was observed in Phos-Tag gel (Fig. 1B, compare lane 4 with lane 1). Phos-Tag gel is used to separate phosphorylated proteins from those unphosphorylated (45). Yki Ser-168 is reported to be phosphorylated by Wts kinase; to figure out whether the entire Yki mobility shift band in Phos-Tag gel resulted from Wts-mediated phosphorylation, we repeated the above experiment using Yki-3SA whose Wts-mediated phosphorylation sites (Ser-111, Ser-168, and Ser-250) were mutated to alanine (46). As shown in Fig. 1B, coexpression of CK2 with Yki-3SA failed to induce shift band of Yki-3SA in Phos-Tag gel, suggesting that CK2 changes the phosphorylation status of Wts-targeted Yki sites.
We also found that the increase of Yki phosphorylation is in response to CK2 kinase activity, as the CK2 kinase dead form (a lysine to arginine mutation at the 66 site of CK2/H9251 subunit) failed to induce either Ser(P)-168 increase or Yki mobility shift in Phos-Tag gel (Fig. 1, A, lane 3, and B, lane 6). Moreover, neither CK2α expression nor CK2β expression was able to induce a dramatic Yki phosphorylation compared with the CK2 holoenzyme (Fig. 1B, compare lanes 2 and 3 with lane 4), suggesting CK2-induced Yki phosphorylation requires co-expression of both CK2α and CK2β subunits.

Yki activity is tightly controlled by Hpo signaling via phosphorylation as well as protein-protein interactions (30). Wts phosphorylates Yki to induce binding between Yki and 14-3-3 protein to restrict Yki in cytosol and hence inhibits Yki activity. Because CK2 promotes Yki phosphorylation on Wts-targeted Yki sites, we sought to confirm whether the CK2-promoted Yki phosphorylation truly affects Yki activity. A cell fractionation experiment was performed to separate the nucleus from cytoplasm, and the distribution of Yki was examined by Western blot analysis (Fig. 1C). In normal cellular conditions, Yki localizes predominantly in cytoplasm. Coexpression of CK2 moderately increases Yki cytoplasmic distribution (Fig. 1C). We also noticed that Scalloped coexpression-induced Yki nuclear translocation was significantly affected by CK2 coexpression. As expected, coexpression of CK2KD was unable to drive Yki out of the nucleus, suggesting that CK2 kinase activity is necessary for its function on driving the Yki nuclear-cytoplasmic shuttle. In addition to the detection of Yki localization change, we checked the dynamic interaction between Yki and 14-3-3 protein (17) by co-immunoprecipitation experiments. The binding between Yki and 14-3-3 protein is enhanced when CK2, but not CK2KD, was coexpressed (Fig. 1D) although not as strong as Hpo/Wts coexpression-induced binding. Taken together, we provide evidence that CK2 promotes Yki phosphorylation and cytoplasmic localization to inhibit Yki activity.
FIGURE 2. CK2 promotes Wts to phosphorylate Yki. A, CK2-induced Yki phosphorylation is dependent on Wts but not Hpo. S2 cells expressing the indicated constructs were treated with dsRNA targeting different genes and probed with indicated antibody. Relative mRNA level of hpo and wts in different samples were quantified by real-time PCR. The experiment was repeated three times, and representative blots are shown. B, GST-Yki protein was incubated with immunoprecipitated (IP) Wts-V5 and then probed with GST or Ser(P)-168 antibody. In different samples Wts was coexpressed with different combinations of CK2 subunits. The experiment was repeated three times, and representative blots are shown. C, GST-Yki protein was incubated with immunoprecipitated FLAG-CK2 or Wts-V5 and probed with GST or Ser(P)-168 antibody. Note that only when incubated with Wts could Yki be phosphorylated. The experiment was repeated three times, and representative blots are shown. D, knockdown of CK2 decreases Wts activity to phosphorylate Yki. S2 cells expressing the indicated constructs were treated with dsRNA targeting different genes and probed with indicated antibody. Relative mRNA levels of CK2α and CK2β in different samples were quantified by real-time PCR. The experiment was repeated three times, and representative blots are shown.
CK2 Enhances Wts-induced Yki Phosphorylation—To dissect the mechanism of how CK2 inhibits Yki activity, we tested whether CK2 directly phosphorylates Yki as it is a serine-threonine kinase. Considering that CK2 promotes Yki phosphorylation on Wts-targeted serine sites, we cotransfected Yki with CK2 in S2 cells with treatment of wts dsRNA to avoid the interference induced by Wts-induced Yki phosphorylation. The efficiency of wts dsRNA treatment was confirmed by mRNA quantification (Fig. 2A). To our surprise, we found that CK2 no longer induced Yki phosphorylation upon Wts depletion (Fig. 2A, compare lane 4 with lane 2), indicating that CK2 only promotes Wts-induced Yki phosphorylation rather than directly phosphorylates Yki.

To figure out how CK2 promotes Wts-induced Yki phosphorylation, we first coexpressed CK2 and Yki with Hpo depletion. Compared with Wts knockdown, Hpo knockdown decreased but not completely abolished CK2-promoted Yki Ser-168 phosphorylation (Fig. 2A, compare lane 3 with lane 2), indicating that Hpo is not necessary for CK2-promoted Yki phosphorylation. To confirm the efficiency of hpo dsRNA treatment, the hpo mRNA level was quantified by real-time PCR (Fig. 2A). Moreover, we examined the effects of Ex depletion or Tao-1 depletion and found that neither blocks the CK2-promoted Yki Ser-168 phosphorylation.4 These results suggest that CK2 promotes Yki phosphorylation possibly through enhancing Wts activity but independent of components upstream of Wts in Hpo signaling.

To further confirm the function of CK2 on Wts-induced Yki phosphorylation, we carried out kinase assay using in vitro purified GST-Yki protein. When incubated with immunoprecipitated Wts from S2 cell lysates, GST-Yki was phosphorylated on Ser-168 (Fig. 2B, lane 2). We found that coexpression of CK2 and Wts significantly enhanced Wts-induced Ser-168 phosphorylation, which was not observed when CK2KD or each single CK2 subunit was coexpressed (Fig. 2B, compare lane 5 with lane 3, 4, 6, and 7), consistent with our findings in Fig. 2A. However, no matter if Wts was coexpressed or not, if Yki was incubated with immunoprecipitated CK2 in the kinase assay, Ser-168 was not phosphorylated (Fig. 2C), supporting our findings that CK2 does not directly phosphorylate Yki Ser-168 but promotes Wts-mediated Yki phosphorylation. Consistently, CK2 knockdown decreases Wts-mediated Yki Ser-168 phosphorylation. As shown in Fig. 2D, depletion of CK2α decreases Yki phosphorylation levels, whereas CK2β knockdown does not make dramatic effects. In toto, we conclude that CK2 induces Yki phosphorylation through promoting Wts activity.

Drosophila CK2 Is Essential for Tissue Growth—CK2 is known as a cell death suppressor in mammalian systems, whereas interestingly, we found that CK2 promotes Wts-induced Yki phosphorylation to suppress Yki activity. To address why CK2 suppresses Yki activity while it functions as a cell death suppressor, we used CK2 transgenic flies and CK2 RNAi flies for in vivo functional analyses. Although both Drosophila ck2α and ck2β null alle as are available (47, 48), ck2α locates at chromosome 80D1, which is hard to recombine with FRT80, and as ck2β mutant clone is too small for analysis, we were not able to generate suitable CK2 mutant flies for loss-of-function analysis. Therefore, we used CK2RNAi flies for loss of function analysis.

To understand the function of CK2 in Drosophila development, we examined the adult phenotype of flies overexpressing CK2 RNAi. Flies overexpressing CK2α RNAi by eyeless-Gal4 produced smaller adult eyes compared with wild type flies (Fig. 3, A and B), whereas overexpression of CK2β RNAi led to adult lethal. Furthermore, we found that knockdown of CK2α, CK2β, or both by RNAi with MS1096-Gal4 resulted in the formation of small wings (Fig. 3, C–F). Of note, the phenotype of CK2βRNAi expression is more severe than CK2αRNAi expression. This phenomenon may result from a difference between RNAi efficiencies of CK2α and CK2β or may be because CK2β not only binds to CK2α but also forms complex with other proteins (36). To this end, CK2 RNAi efficiency was confirmed

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CK2 influences Yki activity

We have shown that CK2 is required for tissue growth, which seems to go against our previously finding that CK2 promotes Yki Ser-168 phosphorylation. Therefore, we checked whether CK2 expression level was manipulated. Besides diap1, other well known Yki target genes include Ex, an upstream Hpo pathway component (19), and microRNA bantam, which controls cell proliferation and apoptosis (51, 52). We noticed that although CK2 overexpression up-regulates Diap1 protein level, it barely affects the reporter genes diap1-GFP4.3 and Ex-lacZ (Ex-Z), which represent transcriptional levels of Diap1 and Ex, respectively. These reporters are frequently used to reflect Yki activity (19, 31). Such results suggest that, although CK2 overexpression induces up-regulation of Diap1 protein level in vivo, it hardly influences Hpo signaling as the transcriptional level of Yki targets shows no change.

We then examined the effect of the loss-of-function of CK2 on Hpo signaling. CK2 knockdown by RNAi using hhGal4 induced a severe growth defect as well as down-regulation of Diap1 protein level in the P compartment (Fig. 3, L and L’), indicating an increase of Yki activity in this condition. However, dramatic up-regulation of diap1-GFP4.3 and Ex-Z was observed in the diminished P compartment (Fig. 5, D and E’), indicating an increase of the transcriptional activity of Yki although the P compartment of the tissue underwent apoptosis. Also, a bantam sensor (BS) signal that reversely correlates with bantam expression was down-regulated (Fig. 5, F and F’). In addition, consistent with the up-regulation of target genes, Yki accumulated in the nuclei of the cells expressing CK2RNAi (Fig. 5, G and G’), indicating an increase of Yki activity in this condition. Of note, we noticed that the up-regulation of Yki targets cannot induce growth in this condition. We speculated that CK2 function is essential for Yki-induced cell proliferation. As shown in Fig. 5, H–J, knockdown of CK2 by RNAi suppressed Yki overexpression-induced tissue growth.

**FIGURE 4. Knockdown of CK2 expression by CK2RNAi.** A–C, knockdown of CK2 mRNA by CK2 RNAi. WT or CK2RNAi wing discs were isolated, and the mRNA level of the indicated genes was analyzed by quantitative real time-PCR. D–H, CK2 RNAi-induced diminished wing phenotype could be partially rescued by overexpression of CK2. Adult male wings expressing UAS-CK2RNAi (E), UAS-CK2RNAi+UAS-FLAG-CK2α (F), UAS-CK2RNAi (G), and UAS-CK2RNAi+UAS-FLAG-CK2β (H) with MS1096-Gal4 were compared with wings expressing MS1096-Gal4 (D). Bar = 500 μm. Experiments were repeated, and representative wings are shown.
To further confirm our result, we generated flip-out clones expressing CK2RNAi in developing discs to check the changes of Yki targets. Also, the up-regulation of Diap1-lacZ (Diap1-Z) and Ex-Z and down-regulation of BS were observed clearly in CK2RNAi clones (Fig. 6, A–C’’’). Moreover, the up-regulation of the targets was suppressed when Wts was coexpressed with CK2RNAi (Fig. 6, D–G’), further suggesting this regulation is through Wts. To investigate which subunit of CK2 is more important for the function on Yki activity in vivo, we tested the effect of knockdown of each subunit alone. We found that the activity of CK2βRNAi was much stronger than CK2αRNAi.
as up-regulation of Yki targets can be easily observed in CK2βRNAi clones (Fig. 6, J–K’), whereas the change is not dramatic in CK2αRNAi clones (Fig. 6, H–I’). These findings are consistent with adult wing phenotypes (Fig. 3, C–F).

**CK2 Overexpression Suppresses hpo Mutant-induced Tissue Growth**—Why overexpression of CK2 hardly affect Yki activity? We speculated that Hippo signaling tightly controls Yki activity in vivo so that further inhibition effect induced by CK2 overexpression is faint. However, loss of hpo leads to an inactivation of Wts; thus, the effect of CK2 overexpression on promoting Wts might be clear. We then tried to test this hypothesis under the condition that Yki is hyper-activated by blocking Hpo activity. hpo mutant clones generated by a MARCM (mosaic analysis with a repressible cell marker) technique are with defects of Hpo signaling and the up-regulation of Ex in Drosophila eye discs and led to an eye overgrowth phenotype in adults (Fig. 7, A, A’, and E). We found that the overexpression of CK2, but not CK2KD, in hpo clones suppressed Ex up-regulation (Fig. 7, B and B’), whereas the change is not dramatic in CK2/K/H9251 RNAi clones (Fig. 6, H–I’). These findings are consistent with adult wing phenotypes (Fig. 3, C–F).

**DISCUSSION**

Until now CK2 was reported to be involved in different cellular processes and signaling pathways. Most of the research shows that CK2 is a widely expressed cell death suppressor and...
plays roles in promoting cell survive. In this study, we identified a dual role of CK2 in tissue growth. On the one hand, we showed that Drosophila CK2 is essential for tissue growth through caspase signaling; on the other hand, we provided evidence that CK2 promotes Wts-induced phosphorylation to suppress Yki activity to inhibit growth. Using site-specific antibody and Phos-Tag gel, we found that CK2 overexpression induces Yki phosphorylation on Wts target sites, leading to similar effects of activated Hpo signaling, including Yki nuclear-cytoplasmic translocalization and Yki-14-3-3 interaction (Fig. 1). We further provided genetic evidence that CK2 knockdown up-regulates the expression levels of Yki target genes (Figs. 5 and 6), which is consistent with in vitro findings. However, as CK2 is involved in several signaling pathways including Wnt and Hh pathway (44, 53, 54), it is possible that CK2 also act through these pathways to affect cell survival and Yki activity.

Interestingly, our findings suggest that CK2 induces Yki phosphorylation via promoting Wts activity, as CK2 can no longer change Yki phosphorylation status when Wts was depleted (Fig. 2A). The in vitro kinase assay also shows that Wts but not CK2 directly phosphorylates Yki, and the change in CK2 protein levels affect Wts activity dramatically (Fig. 2, B–D). In the MARCM system, CK2 overexpression suppresses the phenotypes induced by hpo mutant but not by wts mutant, suggesting the suppression of Yki activity by CK2 is dependent on Wts but not Hpo (Fig. 7).

As a key player, Wts is regulated by several components in the Hpo pathway (10, 55, 56). Recently, it is reported that Merlin recruits Wts to the cell membrane where Wts is phosphorylated and activated by Hpo/Salvador (57). Here our findings indicate that CK2 is a novel Wts regulator. What is the difference of CK2 function and the function of other Wts regulators in Wts regulation and how CK2 and other Wts regulators coordinate spatial and temporal are interesting questions in need of further investigation. However, we detected neither Wts-CK2 interaction in co-immunoprecipitation experiment nor phosphorylation shift of Wts by CK2 overexpression; therefore, whether CK2 directly regulates Wts remains unclear.

In this study our works uncover a dual role of CK2. One is that CK2 promotes Wts activity to phosphorylate Yki and hence suppresses Yki activity. The other one is that CK2 is essential for cell survival. Both Yki and CK2 are important in controlling cell proliferation and apoptosis. Our results suggest that CK2 and Yki may affect cell survival via different mechanisms. Although their functions in anti-apoptosis processes are both related with Diap1, Yki regulates Diap1 transcription levels, whereas CK2 affects at protein levels. Knockdown of CK2 induces inhibition of the tissue growth (Fig. 3) but up-regulation of Yki targets (Figs. 5 and 6), implying that CK2 may play roles on suppressing Yki activity. Yet the up-regulation of Yki targets cannot reverse the cell death fate, suggesting the function of CK2 promoting survive is the major role. Moreover, suppression of Yki activity by CK2 was observed in hpo mutant background but not in normal conditions. On the basis of these results, we suppose that the function of CK2 in promoting Wts activity is coordinated with its anti-apoptotic function, which fine-tunes cell proliferation and cell death. Several questions await further investigation. Why does CK2 have a dual role in cell survival? What is the trigger to switch the functions? How does CK2 balance its roles to ensure a normal cell growth? A deep understanding of the underlying mechanism may help us to answer these questions.

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