Chapter 8
Function of Translationally Controlled Tumor Protein in Organ Growth: Lessons from Drosophila Studies

Kwang-Wook Choi, Sung-Tae Hong, and Thao Phuong Le

Abstract Regulation of cell growth and proliferation is crucial for development and function of organs in all animals. Genetic defects in growth control can lead to developmental disorders and cancers. Translationally controlled tumor protein (TCTP) is a family of evolutionarily conserved proteins implicated in cancer. Recent studies have revealed multiple roles of TCTP in diverse cellular events, but TCTP functions in vivo are poorly understood in vertebrate systems. We have used *Drosophila melanogaster*, the fruit fly, as a model organism for genetic dissection of Tctp function. Our studies have shown that Tctp is essential for organ development by regulating growth signaling. Furthermore, it is required for genome stability by promoting DNA repair and chromatin remodeling in the nucleus. Thus, Tctp acts as a multifaceted cytosolic and nuclear factor for regulating organ growth and genome stability. In this chapter, we describe an overview of our findings on Tctp functions in *Drosophila* and discuss their implications in cancer.

8.1 Introduction

TCTP family proteins are widely expressed in eukaryotes. It was first identified in mouse tumor cells in the early growth phase (Bohm et al. 1989; Chitpatima et al. 1988). TCTP is abundantly expressed in rat and human testes, a tissue that undergoes intense mitotic activity (Guillaume et al. 2001). In yeast, TCTP is expressed throughout the cell cycle (Chung et al. 2000), but is greatly upregulated during exponential growth (Norbeck and Blomberg 1997), whereas it is repressed in conditions of growth arrest

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(Bonnet et al. 2000). Furthermore, extensive analysis of differential gene expression in the tumor cells and revertants revealed that TCTP is upregulated in cancer cells derived from different organs and is among the most downregulated genes during tumor reversion, the process of "quitting the malignant phenotype" (Amson et al. 2012, 2013; Bommer and Thiele 2004; Tuynder et al. 2002, 2004). These studies suggest important roles of TCTP in growth regulation, tumorigenesis, and its reversion process.

In addition, TCTPs have been implicated in several other functions, including histamine release (Nielsen et al. 1998), microtubule association (Gachet et al. 1999; Jeon et al. 2016), and Ca^{++} regulation (Kim et al. 2000; Sanchez et al. 1997). An important clue to the molecular function of TCTP was provided from its structure. TCTP family proteins turned out to be related to a guanine nucleotide exchange factor (GEF) for Rab proteins (Dong et al. 2009; Thaw et al. 2001), suggesting that TCTPs might be involved in intracellular vesicle trafficking (Predic et al. 2002). Many of these TCTP functions are based on biochemical interactions and functional assays in culture cells but not in animals. Thus, whether TCTP plays such roles in tissues and organs of animals in vivo is an important question to be addressed.

Drosophila is an ideal system for studying gene functions in vivo. We became interested in Tctp initially by chance (hereafter, *Drosophila TCTP* gene and protein are labeled "*Tctp*" and "Tctp", respectively, according to the FlyBase nomenclature). Our work since then has revealed important functions of Tctp in the regulation of growth and genome stability, providing new insights into the roles of mammalian TCTP genes. Firstly, we will begin with a brief background of our Tctp work. Secondly, we will discuss the function of Tctp in TOR signaling for organ growth. Thirdly, we will present a nuclear function of Tctp for DNA repair and genome stability. Lastly, we will discuss possible implications of our works on mammalian TCTP functions and cancer.

8.2 Identification of *Drosophila* Tctp Function in Organ Growth

Nothing was known about the *Tctp* gene in *Drosophila* when we first noticed its putative role in tissue growth. At the time, we had been studying how the adult compound eye develops from the eye imaginal disc, an epithelial primordium for the eye. In the early stages of eye development, establishment of the dorso-ventral (DV) axes of the eye disc is critically important for its growth and patterning (Singh et al. 2005). While searching for genes involved in the growth of the eye disc, we found a strong genetic interaction between a mutation affecting eye growth and a deficiency chromosome uncovering the *Tctp* locus.

This genetic enhancement of the eye growth phenotype suggested a role of Tctp in organ development. Because there was no known mutation in the *Tctp* gene, we first generated *Tctp* mutants by imprecise excision of a P-element inserted in an intron of the

Tctp gene (Hsu et al. 2007). Genetic tests suggested one of these mutations to be a null allele. This mutant was lethal as homozygotes, resulting in death during early larval stages. Some *Tctp* null homozygotes go through embryogenesis probably due to the presence of wild-type maternal Tctp protein deposited from heterozygous mothers, but they all die during early larval stages. Thus, it is clear that Tctp plays important functions for larval stages during which primordia for adult organs undergo active development.

To understand the function of Tctp in organ development, we mainly used the eye and the wing, two appendages that have been studied extensively (Baker 2007; Cohen and Di Nardo 1993). These organs develop from the eye and the wing imaginal disc, respectively. Although development of these organs involves several conserved signaling mechanisms, the ways these signaling pathways are used for axial patterning and differentiation of the eye and the wing are quite different (Baker 2007). Nonetheless, tissue-specific Tctp knockdown using RNA interference (RNAi) in these developing organs by using Gal4–UAS system (Brand and Perrimon 1993) results in similar growth defects. Because organ growth depends not only on cell proliferation but also on the cell size, it is important to determine whether Tctp is involved in the control of cell size, cell number, or both. Since each cell of adult wing has a single hair protruding from the cell surface, the hair density provides an approximate measure of cell size and number. Using the hair density, we showed that both cell number and size are reduced when Tctp is partially depleted by RNAi (Hsu et al. 2007), indicating the requirements of Tctp for cell growth as well as proliferation.

To support the results of Tctp RNAi phenotypes in organ growth, *Tctp* null mutant was also examined using genetic mosaic clones. Because *Tctp* null mutant flies die during early stage of development, we utilized methods of making genetic mosaic animals in which patches of homozygous *Tctp* mutant clones can be generated in heterozygous flies by mitotic recombination (Xu and Rubin 1993) (Fig. 8.1a). The clone size depends on the number of cell division after the first mitotic recombination event. When *Tctp* mutant clones were examined in wing discs soon after mitotic recombination was induced at first instar larval stage, both +/+ wild-type twin spots and $Tctp^{-}/Tctp^{-}$ mutant clones were very small but showed similar sizes (Fig. 8.1b). On the contrary, when mosaic wings were examined during late third instar stage, $Tctp^{-}/Tctp^{-}$ clones remained small or were eliminated while +/+ twin spot clones had grown much faster, eventually competing out $Tctp^{-}/Tctp^{-}$ mutant cells (Fig. 8.1c). This clonal analysis demonstrated that Tctp is essential for growth of tissues in wing discs. Another interesting point is that *Tctp* mutant cells cannot compete well with adjacent wild-type cells, eventually being lost, thus indicating the importance of Tctp in cell survival.

8.3 Role of Tctp in TOR Signaling

Clonal analysis of *Tctp* null mutation demonstrated that Tctp is required for organ growth. We tested whether the growth defects of *Tctp* mutant cells are due to abnormalities in cell proliferation and/or cell survival. A genetic technique called

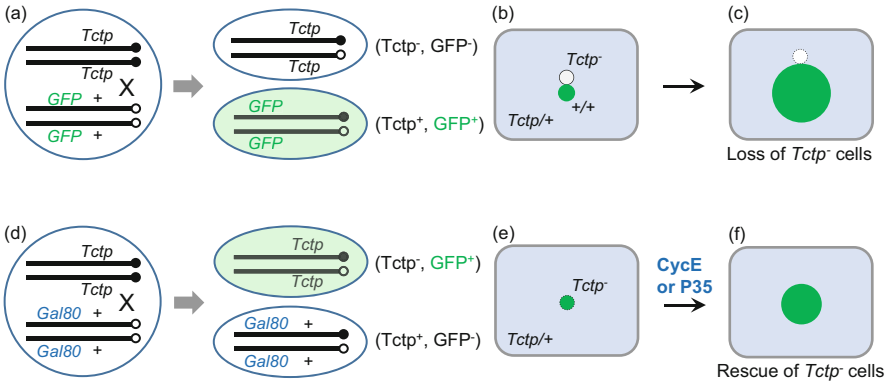

Fig. 8.1 Suppression of *Tctp* mutant defects by CycE and P35. (**a**–**c**) Growth defects of *Tctp* mutant clones in wing disc. (**a**) $Tctp^{-}$ mutant clones are generated by mitotic recombination in $Tctp^{-}$/+ heterozygote cells. $Tctp^{-}$ clones and wild-type twin spots (+/+) are marked by the absence or presence of GFP expression. (**b**) At 24 h after clone induction, both $Tctp^{-}$ and +/+ twin spot clones are small but similar in size. (**c**) By 60 h after clone induction, +/+ clones grow large but most $Tctp^{-}$ clones are eliminated. (**d**–**f**) Partial suppression of $Tctp^{-}$ mutant defects by CycE or P35. (**d**) Clones are generated by the MARCM method. The presence of *Gal80* in $Tctp^{-}$/+ heterozygous wing cells represses Gal4-dependent GFP expression. The presence of *Tub-Gal4* and *UAS-GFP* are not shown for simplicity. After recombination, *Tctp* mutant cells can express GFP due to loss of *Gal80* whereas +/+ twin spot cannot. (e) $Tctp^{-}$ clones are very small or not formed. (**f**) Expression of CycE or P35 in mutant clones partially suppresses growth defects, resulting in the formation of $Tctp^{-}$ clones

MARCM (Mosaic Analysis with a Recessive Cell Marker) (Lee and Luo 2001) provides a powerful in vivo tool for expressing a gene within mutant clones. Using this technique, we overexpressed either Cyclin E (CycE) or P35 caspase inhibitor in *Tctp* mutant clones. Notably, small sizes of *Tctp* null mutant clones were enlarged by expressing cyclin E or P35 in the MARCM mutant clones (Fig. 8.1d–f), indicating that loss of Tctp not only affects cell proliferation but also impairs cell survival (Hsu et al. 2007).

Genetic analysis of *Tctp* mutation and knockdown indicates that Tctp is involved in the regulation of both cell proliferation and cell size. Because the Target of Rapamycin (TOR) signaling regulates both cell growth and proliferation (Laplante and Sabatini 2012b), it was conceivable that Tctp might function in the TOR pathway to control organ growth. A central component of this pathway is TOR protein kinase that phosphorylates S6 kinase (S6k) and Thor (*Drosophila* 4EBP) to promote cell growth (Miron et al. 2001; Oldham et al. 2000). TOR kinase is activated by parallel inputs from growth factor signaling and nutrient conditions (Jewell et al. 2013). Upon insulin receptor (InR) signaling, the GTPase Activating Protein (GAP) activity of the Tuberous sclerosis complex (Tsc1/Tsc2) is inhibited, thus activating Rheb (Ras-related human protein enriched in brain) GTPase and its associated effector TOR (Dong and Pan 2004; Garami et al. 2003; Saucedo et al. 2003; Stocker et al. 2003; Zhang et al. 2000, 2003). Indeed, there was striking genetic interaction between *Tctp* and the TOR pathway genes, including upstream

genes like *Tsc1/2*, *Rheb*, and *InR* as well as a downstream gene *S6k*. For instance, tissue growth induced by overexpression of InR or Rheb can be suppressed by Tctp knockdown (Hsu et al. 2007). Genetic relationships between Tctp and these TOR components suggest that Tctp acts at a step close to Rheb.

In addition to the genetic interaction of Tctp with Rheb, we noted that yeast TCTP/DSS4 (mammalian MSS4) was initially identified as a genetic suppressor of *sec4* gene encoding a Rab GTPase (Burton et al. 1993; Moya et al. 1993). Since TCTP is structurally similar to the Mss4 GTPase regulator (Thaw et al. 2001), Tctp might function in TOR signaling by interacting with Rheb GTPase. Biochemical evidence indicated that Tctp can physically interact with Rheb, and it has a guanine nucleotide exchange (GEF) activity for Rheb. The glutamate residue at the 12th position of TCTP was implicated in binding to Sec4 Rab GTPase (Thaw et al. 2001). We showed that the E to V mutation ($Tctp^{E12V}$) abolished the GEF activity. This residue is also critical for the in vivo function of Tctp because, unlike wild-type Tctp, $Tctp^{E12V}$ fails to rescue the growth defects of Tctp RNAi (Hsu et al. 2007). Taken together, multiple pieces of evidence suggest that Tctp acts through Rheb in order to activate TOR signaling for organ growth (Fig. 8.2a).

The proposed Tctp function as a GEF toward Rheb had been questioned by Wang et al. (2008) and Rehmann et al. (2008) based on the observations that mammalian TCTP could not bind to Rheb and did not reproducibly affect mTORC1 signaling. The cause of these discrepancies on the function of TCTP is not yet clear but is likely to be due to differences in cell cultures and assay conditions. In fact, Dong et al. (2009) demonstrated that human Tctp not only binds to Rheb but also accelerates GDP release from hRheb. Additionally, they showed that hTCTP can prolong the activation of mTOR signaling in amino acid-depleted cells whereas $hTCTP^{E12V}$ mutant form cannot. hTCTP also acts upstream to Rheb for the activation of S6k phosphorylation. All of these results were consistent with the Tctp–Rheb relationships shown in *Drosophila*. In addition, analysis of the structure model of the hRheb–hTCTP complex showed that hTCTP binding to hRheb opens the nucleotide binding site to facilitate the dissociation of GDP. Moreover, key residues involved in the hTCTP–hRheb interaction were experimentally validated (Dong et al. 2009), supporting the function of TCTP as a GEF for Rheb.

The binding between TCTP and Rheb has also been shown in *Arabidopsis* (Brioudes et al. 2010), indicating that this interaction is conserved in invertebrates, vertebrates, and plant systems. We have shown that human TCTP can fully rescue the growth defects in Tctp-depleted *Drosophila* organs (Hsu et al. 2007). Remarkably, *Drosophila* Tctp can also restore the defects in *Arabidopsis TCTP* mutants (Brioudes et al. 2010). These studies suggest strong structural and functional conservation of TCTP family genes among plants, invertebrate animals, and humans.

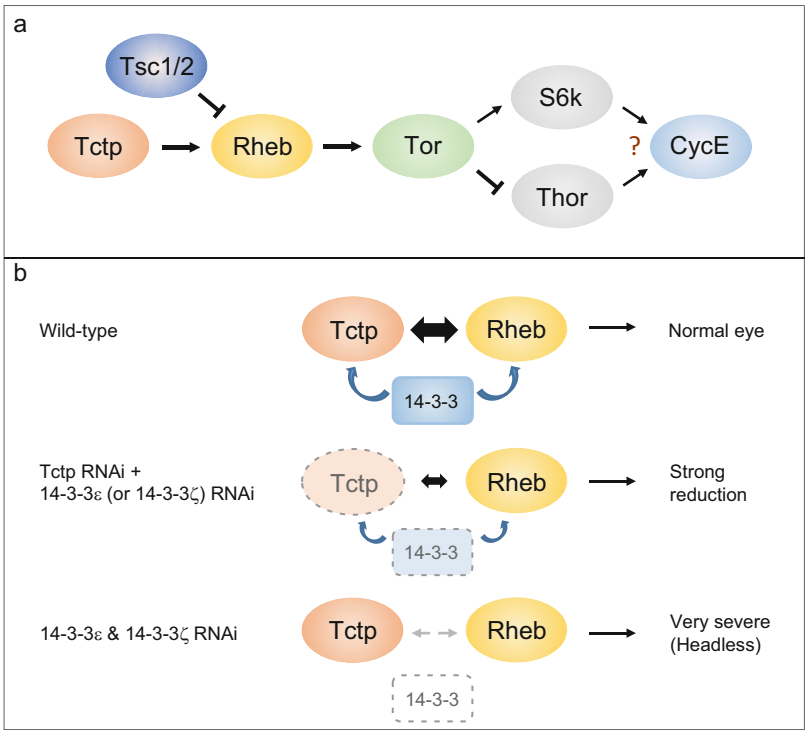

Fig. 8.2 14-3-3 promotes Tctp–Rheb interaction. (**a**) Tor kinase in TORC1 is activated by Rheb GTPase. Tor phosphorylates S6k and Thor/4EBP. Phosphorylated S6k promotes protein synthesis for cell growth. Phosphorylated Thor/4EBP cannot inhibit the function of eIF4, thereby increasing protein synthesis. Increased translation leads to expression of cell cycle regulators. Rheb activity is inhibited by TSC1/2 while facilitated by Tctp. (**b**) In wild-type condition, 14-3-3 isoforms directly interact with Tctp and Rheb, resulting in normal eye size. Tctp RNAi causes a reduction in the eye size. Knockdown of either 14-3-3ε or 14-3-3ζ has no effect, but it strongly enhances the Tctp RNAi eye phenotype. Knockdown of both forms of 14-3-3 abolishes the Tctp–Rheb interaction and disrupts eye disc development causing the headless phenotype. It is unknown whether homo- or hetero-dimerization of 14-3-3 isoform directly links Tctp and Rheb

8.4 Regulation of Tctp Function by 14-3-3

Our study described above suggests that Tctp facilitates the Rheb function in vivo for TOR signaling. Because growth signaling must be precisely controlled for normal development, it is an intriguing question how Tctp function is regulated during organogenesis. In an effort to identify factors that modulate the function of Tctp, we have performed a genetic screen using the Gal4-UAS system (Brand and Perrimon 1993) for targeted knockdown of gene(s). This screen was based on the fact that Tctp RNAi in the eye disc using *eyeless (ey)-Gal4* driver results in a reduction of the eye size. We screened a library of UAS-dsRNA (RNAi for short)

lines to identify specific RNAi lines that either enhance or suppress the Tctp RNAi eye phenotype.

From this screen, we found many RNAi lines that modify the Tctp RNAi eye phenotype. Tctp modifier genes identified in this screen belong to diverse categories, including the ones that are involved in growth signaling, cell death, cytoskeleton, and transcription. Interestingly, 14-3-3 RNAi was found as an enhancer of Tctp RNAi. 14-3-3 is a family of highly conserved proteins expressed in all eukaryotic cells. 14-3-3 proteins play important roles as adaptors in multiple signaling pathways (Morrison 2009). 14-3-3 genes have also been implicated in oncogenesis as well as tumor suppression (Aghazadeh and Papadopoulos 2016; Aitken et al. 2002; Zhao et al. 2011). Mammals have several isoforms of 14-3-3 that have redundant functions as well as cell-type specific roles (Aghazadeh and Papadopoulos 2016; Obsilova et al. 2008). *Drosophila* has two genes, 14-3-3ε and 14-3-3ζ (Skoulakis and Davis 1996). Although silencing of either isoform of 14-3-3 in the eye disc does not affect the eye growth, it synergistically enhances the effects of Tctp RNAi, resulting in much smaller eyes than the size of Tctp RNAi eyes (Fig. 8.2b, Le et al. 2016). Strong genetic interaction between Tctp and 14-3-3 isoforms raised a possibility that 14-3-3 might be involved in regulating the Tctp function in TOR signaling.

Previous studies in mammalian cells have also found that 14-3-3 proteins participate in TORC1 signaling by interacting with TSC2 and PRAS40. Both TSC2 and PRAS40 are negative regulators of Rheb and TOR kinase, respectively (Fonseca et al. 2007; Inoki et al. 2002; Li et al. 2002). Upon phosphorylation of TSC2 by Akt, 14-3-3 binds to phosphorylated TSC2 to inhibit the GAP function of TSC2. PRAS40 directly binds to TOR kinase to inhibit its kinase activity. Binding of 14-3-3 to PRAS40 leads to its dissociation from the TORC1 complex, thereby activating the TORC1 activity (Jewell et al. 2013; Morrison 2009). Hence, 14-3-3 proteins promote TOR signaling by inhibiting these negative regulators of TOR signaling. The serine and threonine phosphorylation sites of Akt (S924 and T1518) are conserved in *Drosophila* and mammalian TSC2 proteins. Interestingly, however, a mutated TSC2 with both substitutions of S924 and T1518 with unphosphorylatable alanine is fully functional to inhibit TOR signaling in *Drosophila* (Dong and Pan 2004). Thus, this phosphorylation-dependent 14-3-3 binding is probably not essential for the TSC2 function in *Drosophila*. Moreover, PRAS40 in *Drosophila* regulates fertility but is not required for growth of the fly, despite its importance in TOR signaling in mammalian cells (Pallares-Cartes et al. 2012). It has not been tested in vivo whether loss of 14-3-3 impairs TOR signaling in mammals. Thus, further studies are necessary to determine the importance of 14-3-3 interaction with TSC2 and PRAS40 in vivo.

Due to the fact that TSC2 phosphorylation is dispensable in *Drosophila*, we figured that genetic interaction between 14-3-3 and Tctp is probably independent of 14-3-3 binding to TSC2. Instead, we found that both 14-3-3 isoforms could directly interact with Tctp (Le et al. 2016), raising the possibility that 14-3-3 might promote TOR signaling through a new mechanism by binding to Tctp. Furthermore, 14-3-3 isoforms can physically interact with Rheb protein as well. Because Tctp and Rheb

act together, these proteins seem to function together with 14-3-3 isoforms. One possibility is that 14-3-3 isoforms might be involved in facilitating the interaction between Tctp and Rheb. In testing this possibility, we found that knockdown of both 14-3-3 isoforms abolished the interaction between Tctp and Rheb to an undetectable level in co-immunoprecipitation assays using *Drosophila* S2 cells. However, depletion of either 14-3-3ε or 14-3-3ζ isoform did not significantly affect the Tctp–Rheb interaction, which is correlated with the observation that knockdown of a single 14-3-3 isoform causes noticeable defects in neither eye nor wing. These results indicate that 14-3-3 isoforms are critical for the interaction between Tctp and Rheb, although the two isoforms share redundant roles.

To test the functional redundancy of the 14-3-3 isoforms, we examined the phenotypes of depleting either one or both of the 14-3-3 isoforms in specific tissues. In striking contrast to the single knockdown of either isoform of 14-3-3, knockdown of both 14-3-3 isoforms using *ey-Gal4* results in pupal lethality. *Ey-Gal4* drives the expression of Gal4 in the primordia for both eye and head during the early stage of development. Examination of dead pupae showed relatively normal body parts, but there was specific loss of entire head and eye tissues to which Gal4 expression was targeted (Fig. 8.2b). Knockdown of both 14-3-3 isoforms in developing wing discs also resulted in severe loss of cell proliferation and induction of specific cell death in the targeted wing area (Le et al. 2016). These tests clearly indicate that two isoforms of 14-3-3 can function redundantly, thereby compensating the loss of one isoform. However, based on the findings that 14-3-3ε or 14-3-3ζ null mutations are semi- or fully lethal as homozygotes, respectively (Acevedo et al. 2007), we presume that these two 14-3-3 isoforms cannot be entirely redundant. Thus, 14-3-3ε and 14-3-3ζ isoforms seem to be partially redundant in certain conditions or tissues, while they also have unique functions. Nevertheless, knockdown of both 14-3-3 isoforms critically impairs normal growth of imaginal discs.

The levels of pS6k and pThor are convenient readouts of TOR signaling, as they are phosphorylation targets of TOR kinase. Interestingly, assays in S2 cells have shown that single knockdown of either of the 14-3-3 isoforms causes considerable reduction of pS6k and pThor (4EBP) levels, indicating that each 14-3-3 isoform is required for full TOR signaling (Le et al. 2016). Thus, TOR signaling in S2 cells might be more sensitive to a reduction of 14-3-3 isoforms than it is for developing organ tissues, although the molecular basis for this difference is unknown. Another important question is how 14-3-3 isoforms promote the interaction between Tctp and Rheb. 14-3-3 proteins are known to function as a homo- or a hetero-dimer (Acevedo et al. 2007; Yaffe 2002). Thus, it is plausible that dimerization of 14-3-3 proteins bound to Tctp and Rheb might bring them together to facilitate their direct interaction. Alternatively, 14-3-3 may be involved in modifying the structure and/or subcellular localization of Tctp and Rheb so that they can form a complex to activate Rheb activity. Additional studies are necessary to pinpoint the precise function of 14-3-3 in the formation of Tctp–Rheb complex.

8.5 Effects of 14-3-3 Isoforms and Tctp on Cyclin E

CycE, as a key regulator of the G1-S transition in cell cycle, plays an essential role in cell proliferation. TOR signaling regulates not only cell growth through activation of S6k and 4EBP but also regulates proliferation by promoting cell cycle. Consistent with genetic and physical interaction of 14-3-3 and Tctp, loss of function clones of *14-3-3ε* or *14-3-3ζ* null mutation results in a partial reduction of CycE level in the eye imaginal disc (Le et al. 2016). However, the partial CycE reduction by depleting one 14-3-3 isoform is insufficient to disrupt proliferation or differentiation of retinal cells of eye disc. We have shown that reduced organ size caused by *Tctp* mutation or RNAi can be rescued by CycE overexpression (Hsu et al. 2007). Therefore, it is likely that defects in organ growth caused by double knockdown of Tctp and one of 14-3-3 isoforms might be due to loss of CycE. As expected, organ growth defects caused by reducing both Tctp and 14-3-3 (or knockdown of both 14-3-3 isoforms) were strongly suppressed by CycE (Le et al. 2016).

In *Drosophila*, TOR kinase activates cell proliferation by regulating the level of CycE (Zhang et al. 2000), although it is not clearly defined how activated TOR leads to CycE expression. Phosphorylation of S6k and 4EBP by activated mTOR signaling results in increased protein synthesis, leading to the promotion of cell cycle (Jewell et al. 2013; Laplante and Sabatini 2012b). In mammalian cells, the eIF4E pathway activated by phosphorylation of 4EBP results in increased translation of mRNAs for cell cycle regulators such as CycD1 (Hashemolhosseini et al. 1998; Laplante and Sabatini 2012a; Rosenwald et al. 1995), thus allowing more CycE–Cdk2 complex to promote cell proliferation. *Drosophila* CycD is not only involved in cell cycle progression but also cell growth through an independent pathway (Datar et al. 2006). It is currently unknown whether the regulation of TOR-dependent CycE level in *Drosophila* is mediated by CycD. Interestingly, suppression of human 14-3-3ε inhibits proliferation of cancer cells and tumor growth. 14-3-3ε inhibition suppresses CycE expression while inducing the cell cycle inhibitor $p27^{kip1}$ at the G1 stage, consistent with the antitumor effect of 14-3-3ε inhibition (Gong et al. 2014). This study supports the role of *Drosophila* 14-3-3 in cell cycle progression during organ development. It would be interesting to see whether the effects of human14-3-3ε inhibition are at least in part mediated by TCTP/Rheb-dependent TOR signaling.

8.6 Tctp Function in DNA Damage Control

TCTP is known to be expressed in the cytosol and the nucleus of normal as well as cancer cells. Although the roles of TCTP in the nucleus have not been extensively studied, it has been reported that TCTP can function as a transcriptional regulator to induce *oct4* genes for the maintenance of stem cell fate (Cheng et al. 2012; Koziol

et al. 2007). These findings suggest that TCTP can have important nuclear functions distinct from its known functions in the cytosol.

In *Drosophila*, first clue to the potential nuclear function of Tctp was provided by the finding that Tctp directly interacts with Ataxia Telangiectasia Mutated (ATM). Previously, we have used a phage display method to identify specific peptide ligands that bind to Tctp. Screening of aptamer libraries helped identify Tctp-binding peptide sequences. ATM was one of the proteins that contain Tctp-binding aptamer sequences and was confirmed to bind Tctp (Hong and Choi 2013). ATM is a serine–threonine protein kinase pivotal for repairing DNA damages caused by double strand break (DSB). Importantly, Tctp was found to form nuclear foci colocalized with ATM upon γ-ray irradiation, which is consistent with its function in DNA damage response. Mammalian ATM forms a protein complex with other factors such as Mre11 and Rad50 for DNA repair. *Drosophila* Tctp was also found to be associated with Mre11 and Rad50, suggesting that Tctp is a member of the functional DNA repair complex. In fact, *Tctp* mutants were defective in various DNA damage responses, showing frequent aberrant chromosomes, abnormal G2/M checkpoint response, and higher cell death upon ionizing irradiation. Consistent with the physical interaction between Tctp and ATM, defective DNA damage responses in *atm* mutants including abnormal chromosomes were strongly enhanced by reducing the level of Tctp. Likewise, growth defects by Tctp RNAi were strongly enhanced by reduced dosages of *atm* and other known DNA damage control genes. Together with the physical interaction between Tctp and ATM, these genetic interactions support the direct involvement of Tctp in DNA damage control in vivo.

Upon sensing DSBs, ATM kinase phosphorylates H2Av (H2AX in mammals), a variant form of histone 2A. The generation of phosphorylated H2Av (γH2Av) is an initial step for recruiting DNA repair proteins. Thus, γH2Av is a biomarker for DSBs and the sites of damage repair foci (Kuo and Yang 2008). Larval salivary gland cells undergo endocycling without mitosis and have a high level of γH2Av. In *Tctp* mutants, levels of γH2Av in salivary glands were diminished compared with the wild-type level. The lower level of γH2Av was rescued by adding a wild-type *Tctp* gene in the mutant background. Furthermore, the level of γH2Av induction upon γ-irradiation was also significantly lower in *Tctp* mutant wing discs than wild-type discs. These results provide in vivo evidence that Tctp is required for repairing both endogenous and exogenous DNA damages.

An important question is how the interaction between Tctp and ATM leads to DSB repair. Since *Tctp* mutations result in a reduction of the γH2Av level, Tctp might be involved in the promotion of ATM kinase activity. In vitro assays showed that addition of Tctp increased the ATM kinase activity toward the H2Av substrate in a dose-dependent manner. Thus, Tctp is directly involved in enhancing the ATM kinase activity, facilitating the DSB repair process. It is worth noting that defective Tctp with E12V substitution could not activate ATM kinase activity, indicating that the E12 residue is critical for the function of Tctp in TOR signaling as well as for the interaction with ATM. Remarkably, human TCTP is also associated with ATM and several proteins involved in DSB repair such as Ku70/80, DNA-binding

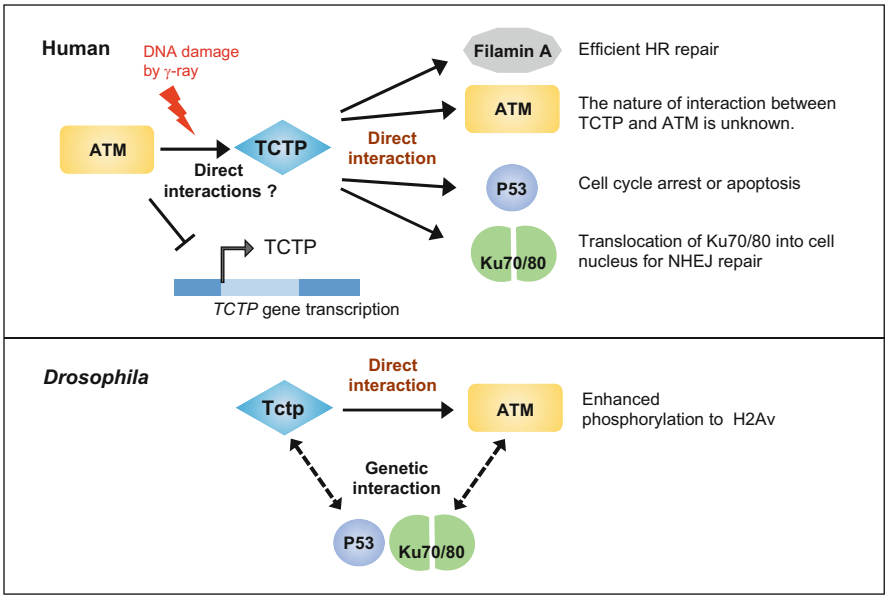

Fig. 8.3 Tctp–ATM interaction for DNA repair in mammals and fly. Relationships between TCTP and ATM in DNA damage response in humans and fly. In humans, ATM inhibits TCTP expression in nonirradiated cells. DNA damage by γ-ray irradiation leads to an increase in the TCTP level through ATM. TCTP forms a protein complex with Filamin A, P53, and Ku70/80 for DNA repair. It is unknown whether TCTP directly binds to ATM. In *Drosophila*, γ-irradiation has little effect on the Tctp level. Tctp directly binds to ATM and promotes its kinase activity. Tctp shows genetic interaction with P53 and Ku70/78, but it is unknown whether this interaction is direct

subunits of DNA-dependent protein kinase. Knockdown of TCTP impairs its ability to repair DSBs in irradiated human cells (Zhang et al. 2012). Thus, *Drosophila* and human TCTP proteins show strong conservation in their nuclear functions for DNA damage response (Fig. 8.3).

We have mentioned earlier that growth defects of *Tctp* mutant cell clones can be partially suppressed by providing CycE as well as the P35 cell death inhibitor, suggesting that Tctp is also required for preventing cell death. This is an important point because mammalian TCTP proteins are anti-apoptotic (Telerman and Amson 2009). One of the mechanisms of TCTP's anti-apoptotic function is related to its ability to bind Bcl-2 family proteins Bcl-xL and MCL1 that inhibit the pro-apoptotic activity of Bax, located in the outer mitochondrial membrane (Liu et al. 2005; Susini et al. 2008). A recent study has shown that TCTP contains a BH3-like domain that recognizes the BH3 domain of Bcl-xL to activate the anti-apoptotic function of Bcl-xL (Thebault et al. 2016). Interestingly, *Drosophila* Tctp also has a putative BH3 domain with conserved hydrophobic residues. In addition to the TCTP interaction with anti-apoptotic mitochondrial proteins, mammalian TCTPs are known to inhibit p53 tumor suppressor-induced cell death upon DNA

damage (Amson et al. 2011; Kloc et al. 2012). TCTP not only represses transcription of p53 but it also destabilizes p53 in order to inhibit apoptosis (Rho et al. 2011). We have shown that reduced eye caused by Tctp RNAi can be suppressed by overexpression of dominant-negative p53 (Hong and Choi 2013). This suggests that cell death resulting from Tctp knockdown is due in part to p53 upregulation or activation and that the relationship between p53 and TCTP in mammalian systems might also be conserved in *Drosophila*.

8.7 Tctp in Chromatin Remodeling and Genome Stability

A closer examination of the nuclear localization of Tctp in the salivary gland indicates that it is associated with most interband regions of polytene chromosomes. Furthermore, from a yeast two-hybrid screen, Tctp was found to interact with Brahma (Brm), the *Drosophila* homolog of yeast Swi/SNF chromatin remodeling factor (Hong and Choi 2016). These findings raised the possibility that Tctp may have a regulatory role at the level of chromatin. A series of genetic and biochemical tests revealed that Tctp is required for inhibiting the ATPase activity of Brm and therefore antagonizing the Brm function in developing organs. Consistent with the role of the Brm remodeler complex in transcriptional gene regulation, loss of Tctp increases RNA polymerase II activity, enhancing transcription in a number of genes.

Interestingly, a reduction in the Tctp level leads to dramatic increases in the transcriptional expression of retrotransposons inserted in the pericentromeric regions, situated near the centromere and highly modified by heterochromatin marks (Hong and Choi 2016). Silencing of retrotransposons by heterochromatin marks is important for the maintenance of genome stability (Larson et al. 2012; Peng and Karpen 2007, 2009; Shi et al. 2008). In addition to its role for inhibiting transposon expression, Tctp is necessary to maintain the stability of *rDNA* genes and other repeated sequences. Methylation of histone 3 at lysine 9 (H3K9me2/3) by SU(VAR)3–9 histone methyl transferase (HMT) is a critical mark for HP1a-dependent heterochromatin formation and gene silencing. Our data demonstrate that Tctp is required for transcription of *su(var)3–9*, hence affecting the levels of H3K9 methylation and HP1a protein (Fig. 8.4).

Position effect variegation (PEV) is a genetic phenomenon caused by repression of the genes abnormally transposed near heterochromatic regions. *Tctp* mutations suppress various PEV phenotypes, consistent with its role in promoting the gene silencing effects of heterochromatin. Tctp and Brm also show the opposing relationship in their effects on PEV. These observations support that the antagonistic relationship between Tctp and Brm contributes to the regulation of the chromatin boundary between euchromatin and heterochromatin (Hong and Choi 2016).

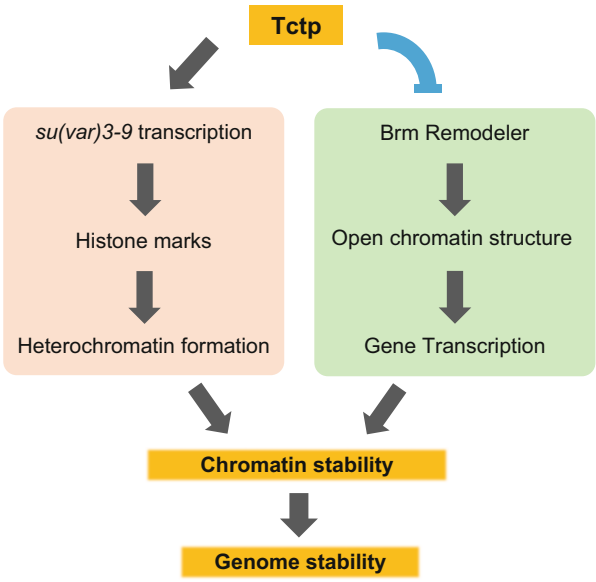

Fig. 8.4 A model for Tctp function in chromatin remodeling and genome stability. Tctp keeps genome stability through two different compaction mechanisms: (1) Heterochromatin formation by inducing transcription of *su(var)3–9*, thus increasing H3K9 methylation at the initial step of chromatin compaction and (2) maintenance of the proper level of chromatin opening by negatively modulating the Brm chromatin remodeler

8.8 Concluding Remarks

Tctp is an evolutionarily conserved protein. Most of structural features of mammalian TCTP family proteins are shared in invertebrate systems like *Drosophila*. TCTP family proteins also seem to be conserved in their in vivo functions, based on the successful complementation of mutant phenotypes by TCTP transgenes from other species. Despite the structural and functional conservation, however, there are several differences between the *Drosophila* Tctp and vertebrate TCTP proteins.

Firstly, TCTP is known as a histamine releasing factor (HRF) that is secreted to promote immune responses (MacDonald et al. 1995). TCTP homologs are secreted from the parasite *Plasmodium falciparum* that causes malaria and found in the plasma of infected hosts (MacDonald et al. 2001). Evidence suggests that TCTP is secreted via exosomal secretory pathway (Amzallag et al. 2004; Lespagnol et al. 2008). It is an intriguing question whether secretion is a general property of TCTP family proteins. If *Drosophila* Tctp can be secreted to act at a distance, phenotypes of *Tctp* mutant clones in genetically mosaic tissues might be rescued by Tctp protein secreted from the adjacent wild-type cells. Thus far, we have not noticed any obvious sign of non-cell autonomous function of Tctp in developing *Drosophila* tissues. It has been shown that truncation of the N terminal sequence and dimerization of TCTP is necessary for its cytokine-like extracellular activity (Kim et al. 2009). A critical cysteine residue of human TCTP involved in the dimerization is also conserved in *Drosophila* Tctp. Therefore, although the function of Tctp in organ growth seems to be cell-autonomous, we cannot exclude the possibility that Tctp can act as a secreted factor in *Drosophila*.

Secondly, one of the most unexpected findings from our *Drosophila* Tctp studies is the apparent lack of gain-of-function Tctp phenotypes. Human TCTP is upregulated in various cancer cells (Tuynder et al. 2002) and has been implicated in tumorigenesis (Bae et al. 2015; Jung et al. 2011; Niforou et al. 2008). However, despite the critical requirements of *Drosophila* Tctp for organ growth, ectopic or overexpression of Tctp in various tissues does not induce tumorous overgrowth (Hong and Choi 2013). Thus, Tctp functions seem to be permissive rather than instructive. It is unclear why Tctp overexpression does not induce overgrowth in *Drosophila*. One possibility is that upregulation of TCTP observed in various cancer cells might be a secondary consequence rather than the primary cause of cancers. Because TCTP is necessary for cell proliferation and survival, knockdown of TCTP might block the growth of cancer cells, possibly explaining the phenomenon of tumor reversion. Alternatively, although *Drosophila* Tctp overexpression may not be sufficient to induce tumorous growth in normal tissues, it might trigger overgrowth in the presence of an additional factor(s) or under certain cellular conditions. If that is the case, systematic searches for such factors would be critical to understand the mechanism for TCTP-induced tumorigenicity. Studies with transgenic TCTP mice have shown that overexpression of TCTP causes hypertension but with normal appearance at any age (Kim et al. 2008). Therefore, mammalian TCTP may also require additional factors to induce cancer, and it is an interesting possibility that upregulation of TCTP might predispose normal cells to be transformed when combined with an additional factor.

Thirdly, the role of Tctp in DNA damage repair seems to be conserved, but important differences have also been noticed (Fig. 8.3). Both *Drosophila* Tctp and human TCTP are involved in two distinct mechanisms of DNA repair: homologous recombination (HR) and nonhomologous end-joining (NHEJ). In human cells, depletion of ATM in nonirradiated cells results in an increase of TCTP level. Further, low dose γ-rays upregulate TCTP to protect against DNA damage, and this upregulation depends on ATM (Zhang et al. 2012). Thus, ATM seems to be involved in regulating the level of TCTP expression and/or stability in nonirradiated cells or cells exposed to low dose γ-irradiation. In contrast, the level of *Drosophila* Tctp is not influenced by irradiation or ATM function (Hong and Choi 2013). Instead, Tctp directly promotes the ATM kinase activity to phosphorylate H2Av. Therefore, loss of Tctp results in a reduction of γH2Av level. This is also in contrast to TCTP-depleted human fibroblast cells in which the number of γH2AX nuclear foci and the level of γH2AX remains high. Human fibroblast cells might have a mechanism to compensate the loss of TCTP to maintain the γH2AX levels. It is also possible that upregulated human TCTP by ATM might be able to activate ATM kinase activity as in the case for *Drosophila*. Future studies are necessary to understand the basis for these variations in the functional relationship between TCTP and ATM in different organisms.

There is profound evidence that many cancers are associated with genome instability. Because loss of TCTP function impairs DNA repair, it may lead to genome instability causing cancer. Therefore, not only upregulation of TCTP but also its loss may be associated with cancer through distinct mechanisms.

Furthermore, *Drosophila* Tctp is critical for genome stability by regulating global gene expression and chromatin modification. It is an important question to be addressed in the future whether mammalian TCTP might play similar roles in the regulation of chromatin modification and genome stability.

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