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Commentary & View

To Cease or To Proliferate

New Insights into TCTP Function from a Drosophila Study

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KEY WORDS

TCTP, Tor pathway, Rheb, TSC, GEF

ABBREVIATIONS

dTCTP	Drosophila TCTP
Rheb	Ras homolog enriched in brain
GAP	GTPase activating protein
Tor	target of rapamycin
TSC2	tuberous sclerosis complex 2

Addendum to:

Drosophila TCTP is Essential for Growth and Proliferation Through Reculation of dRHeb GTPase

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ABSTRACT

Tor (target of rapamycin) pathway underlies a major signaling mechanism for controlling cell growth and proliferation.[1] Rheb (Ras homolog enriched in brain) is a small GTPase in the Tor pathway.[2-4] Similar to other small GTPases, Rheb cycles between a GTP-bound active state and a GDP-bound inactive state. *TSC2* (tuberous sclerosis complex 2), a gene mutated in an autosomal dominant disease tuberous sclerosis, was shown to be the Rheb-GAP (GTPase activating protein).[5,6] However, a guanine nucleotide exchange factor (GEF) for Rheb had been missing. Human TCTP (translationally controlled tumor protein) has been implicated in cancer, but its function in vivo has not been clearly elucidated. Recently we reported a molecular genetic characterization of TCTP function in Drosophila.[7] Drosophila TCTP (dTCTP) displays GEF activity to Rheb and is essential for Rheb activation in organ growth. Thus, our study provides a tight linkage of dTCTP to the Rheb-TOR pathway. In this addendum, we will briefly overview our findings and discuss our perspectives for future research on TCTP.

TCTP is a highly conserved protein identified about 20 years ago as a translationally controlled protein P21 (or P23) enriched in tumor cell lines.[8] Recently, this protein has drawn special interests because of its potential roles in tumorigenesis. TCTP is not only upregulated in a number of tumor cell lines but also downregulated during tumor reversion.[9,10] TCTP has also been implicated in a variety of intracellular and extracellular functions, including microtubule stabilization, cell cycle, apoptosis, and cytokine release.[11-16] However, these functions of TCTP have been inferred mainly from biochemical interactions and cell culture studies.

To address the function of TCTP in vivo, we took a loss-of-function approach using Drosophila as a genetic model. Reduction of dTCTP by tissue-specific RNA interference (RNAi) or loss-of-function mutations resulted in smaller organs with reduction in both cell size and cell number, a phenotype often seen in mutations in Tor or insulin pathway. Our epistatic analysis suggested that dTCTP acts either downstream or in parallel to insulin receptor, TSC1, and dRheb, but upstream of dS6K. Despite the conserved sequence of TCTP proteins in a wide-range of species, TCTP has little similarity to the sequences of other protein families. However, the three-dimensional structure of fission yeast TCTP ortholog reveals similarities with a family of proteins that bind to the nucleotide-free form of Rab GTPases,[17] providing a clue for its potential biochemical function. Consistent with our genetic evidence, our biochemical assays showed that dTCTP could facilitate the GDP/GTP exchange on dRheb both in vitro and in vivo. Our data led us to propose a model (Fig. 1) in which dTCTP regulates the Tor signaling pathway by directly interacting with dRheb GTPase as a GEF.

This study not only provides new insights into the mechanism of dTCTP function in regulation of dRheb activity but also reveals a complexity of dTCTP function in growth regulation. Firstly, *dTCTP* null mutant displays more severe phenotypes than loss of function mutations in the insulin or Tor pathways. For example, while *dTCTP* null mutant clones are eliminated during development, significant portions of *dRheb* and *Tor* null mutant clones can survive to form adult tissues. This argues against the model that *dTCTP* functions only in regulating the Rheb-Tor pathways. Although different maternal contribution of each gene and variations in genetic background might partially account for these differences, it is equally possible that dTCTP functions as a GEF for more than one GTPase targets. Conversely, other GEFs might exist for Rheb, as one small GTPase can be regulated by more than one GEFs.[18,19]

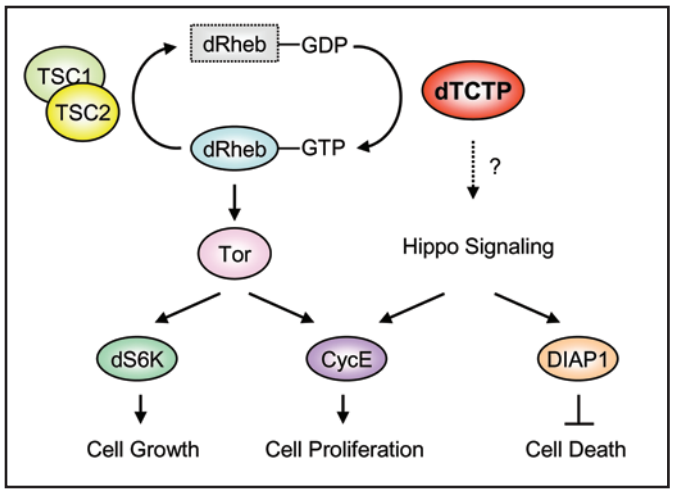

Figure 1. A model for dTCTP function in growth control. dRheb GTPase stimulates Tor signaling, which in turn activates dS6K and CycE to regulate cell growth and proliferation, respectively. dRheb GTPase is inactivated by the GAP function of TSC1/2 complex. In contrast, dTCTP activates dRheb GTPases by promoting GDP-GTP exchange. dTCTP might have additional functions independent of the Tor pathway, such as inhibiting cell death. It remains to be determined if dTCTP regulates cell proliferation and cell survival in part through the Hippo signaling.

Different GEFs might be required for dRheb-TOR function in other developmental events or growth-independent processes like axon guidance during neural development.[20]

Secondly, in contrast to the implicated role of TCTP in cancer,[9] ubiquitous or tissue-specific overexpression of dTCTP was insufficient to cause overgrowth phenotypes in Drosophila. A plausible explanation is that the amount of dTCTP is in excess for dRheb activation in contrast to limited concentrations of TSC2 GAP in normal cells.[5] Since overexpression of dTCTP alone does not result in tumorous overgrowth, it is unlikely that TCTP functions as an oncogene directly under normal condition. However, increased amounts of TCTP in tumor tissues may provide better potency for cells to undergo uncontrolled proliferation and massive overgrowth. In this regard, it will be intriguing to learn if dTCTP can act corporately with other oncogenes. Since the amino acid sequence identity of dTCTP and human TCTP is only 48%, it is also possible that a non-conserved region(s) of human TCTP may be required to induce tumors in human cells. Soon after the publication of our work, knockout of the mouse TCTP was reported. Remarkably, loss of TCTP in mice results in early lethality with smaller sizes of embryos.[21] Developmental defects seen in mutant mice may be analogous to the phenotypes of *dTCTP* mutants in Drosophila, supporting the conserved function of TCTP in growth regulation. However, it is yet to be determined whether there is a common molecular basis for the developmental defects in both systems and whether overexpression of mouse TCTP in transgenic mice can induce tumors.

Lastly, in addition to Tor and insulin signaling pathways, a third pathway consisting of Hippo-Warts protein kinase cascade controls organ size by affecting mainly the cell number.[22,23] The Hippo pathway regulates both cell proliferation and cell death by promoting cyclin E expression and downregulating Drosophila Inhibitor of Apoptosis 1 (DIAP1). It is worthy of note that dTCTP also regulates the cell number by affecting cell proliferation and apoptosis. It remains to be determined whether dTCTP and Hippo signaling pathways crosstalk or are independent of each other.

Studies on TCTP suggest that its function is much more complex than what is known and its interactions with a multitude of proteins might underlie this complexity. Biochemical studies have identified several proteins interacting with TCTP. Given the feasibility of Drosophila genetics, the physiological relevance of these protein interactions can now be addressed in the context of normal development. It would also be powerful to take the advantage of Drosophila genetic screens for the identification of novel genes interacting with *dTCTP*. A more comprehensive understanding of TCTP functions and mechanistic explanations of its intriguing expression profiles in cancers can be expected in years to come.

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