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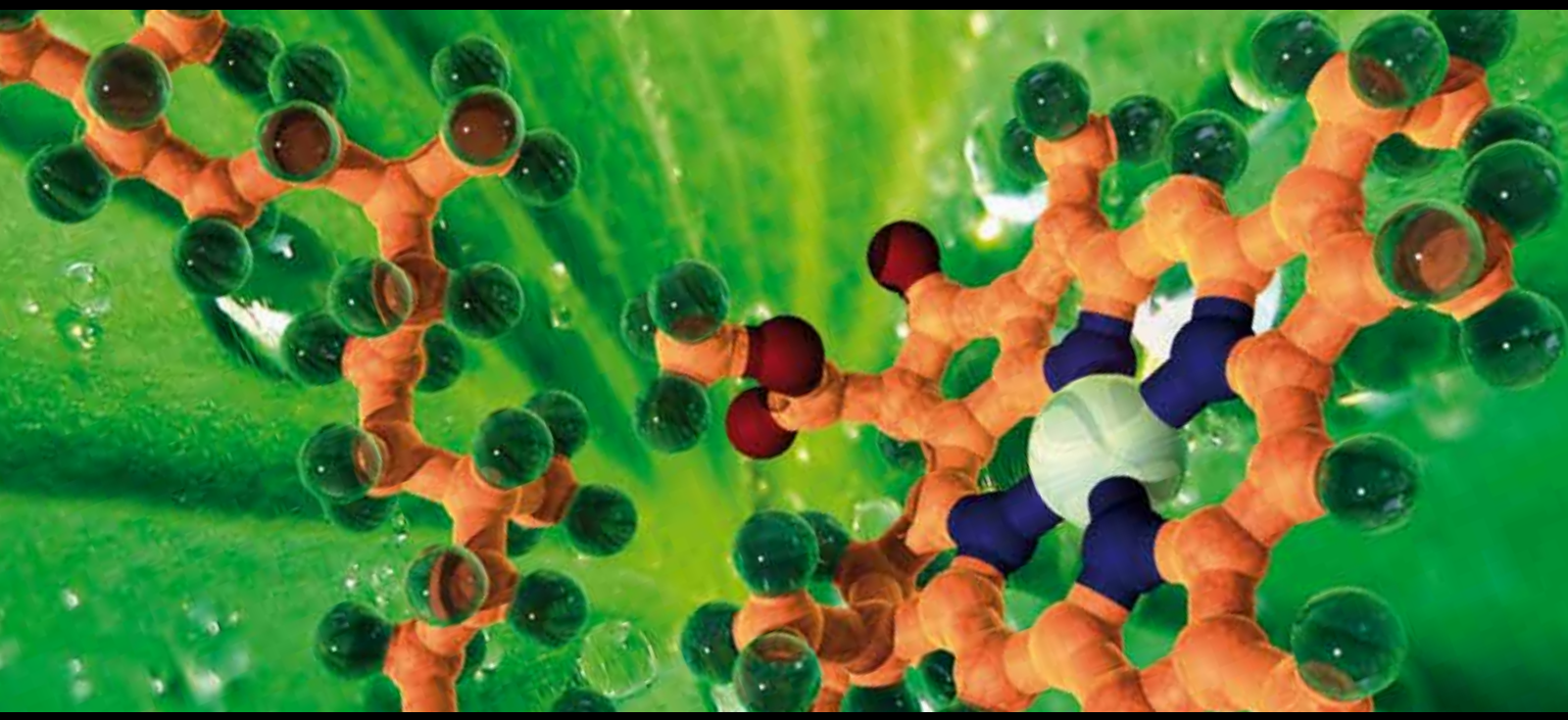
# Translationally Controlled Tumor-Associated Protein

Guest Editors: Malgorzata Kloc, Jacek Z. Kubiak, and Rafik Mark Ghobrial



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# Editorial Board

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*Editorial*

# Translationally Controlled Tumor-Associated Protein

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Translationally-controlled tumor-associated protein (TCTP) has been discovered in 1983 in mouse erythroleukemia cells. Over the years, it became evident that TCTP is an important player in a number of basic cell physiology events in cancer, embryo development, cell cycle, apoptosis, proliferation, growth, stress response, allergy, gene regulation, and heat-shock response. However, despite the nearly three decades of research, we only start to understand the role of TCTP in physiology of animal and plant embryo development as well as in numerous pathologies through its participation in cell cycle, proliferation, and growth regulation. The exact roles of TCTP in many complex cellular processes still remain a mystery. One of the key questions in cancer research is the role of TCTP in tumor reversion, the rare event leading to tumor regression and a "miraculous" cure: is TCTP involved in gene regulation or rather modification of the cytoskeleton of cancer cells during this process? It seems plausible that a novel type of posttranslational modification of TCTP, such as SUMOylation, by regulating its nuclear localization and/or its association with the centrosomes (both subjects featured in this issue) is responsible for some of the TCTP functions in normal and cancer cells. From presented in this issue very comprehensive and up-to-date reviews on TCTP functions, it clearly transpires that TCTP has a potential to be a crucial target for anticancer therapies. However, more research on the regulation of TCTP and its involvement in various molecular and cellular pathways and its association with subcellular structures is needed for the improvement of our understanding of this oncogene and the development of novel TCTP-targeted cancer therapies. We hope that our special TCTP issue will help in stimulation of scientific research in this field.

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*Review Article*

# Biological Effects of Mammalian Translationally Controlled Tumor Protein (TCTP) on Cell Death, Proliferation, and Tumorigenesis

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Translationally controlled tumor protein (TCTP) is a highly conserved protein found in eukaryotes, across animal and plant kingdoms and even in yeast. Mammalian TCTP is ubiquitously expressed in various tissues and cell types. TCTP is a multifunctional protein which plays important roles in a number of cell physiological events, such as immune responses, cell proliferation, tumorigenicity, and cell death, including apoptosis. Recent identification of TCTP as an antiapoptotic protein has attracted interest of many researchers in the field. The mechanism of antiapoptotic activity, however, has not been solved completely, and TCTP might inhibit other types of cell death. Cell death (including apoptosis) is closely linked to proliferation and tumorigenesis. In this context, we review recent findings regarding the role of TCTP in cell death, proliferation, and tumorigenesis and discuss the mechanisms.

## 1. Introduction

Translationally controlled tumor protein (TCTP) was initially identified as a factor implicated in cell growth [1, 2]. TCTP has also been termed histamine releasing factor (HRF), fortilin, P21, P23, TPT-1, and Q23. This protein was named TCTP because its mRNA was controlled at the translational level [3–5]. Although TCTP is found ubiquitously in tissues and cell types, its expression is relatively low in lung and colon, and cell lines derived from normal cells such as a mouse fibroblast NIH-3T3 and human embryonic kidney HEK293T cells [6]. Because of its multifunctional properties, TCTP has attracted the attention of an increasing number of researchers in many fields (reviewed in [7]). TCTP plays important roles in a number of cell physiological events in cancer, cell proliferation, stress response, gene regulation, and heat shock response [8–13]. TCTP was also shown to possess an extracellular function, that is, histamine release [14].

Tumorigenicity, proliferation, and cell death, including apoptosis, are closely related functions. Uncontrolled or promoted proliferation and loss of cell death are general properties of tumor cells. In this paper, we will focus on mammalian TCTP and discuss its physiological functions, emphasizing cell death, proliferation, and tumorigenesis.

## 2. Properties of TCTP

Human [5] and murine [3] TCTP cDNAs were isolated and their sequences determined more than 20 years ago. Human TCTP cDNA encodes a protein with a calculated molecular mass of 19 kDa (172 amino acids). Sequence analyses revealed that TCTP is a highly conserved protein lacking homology to any other protein. TCTP has been found in a wide range of eukaryotes, including yeast, plants, and animals, suggesting it originated in the distant evolutionary past. Since immune systems are restricted to animals, its function in histamine release has been acquired only recently in evolution. Ubiquitous expression of TCTP in mammalian tissues suggests its importance in normal physiological functions. In fact, a gene-targeting approach

revealed that TCTP is an essential protein in mice since knockouts deficient in this protein die at embryonic stage day E9.5-E10.5 [15]. However, studies with mouse embryonic fibroblast (MEF) cells showed that TCTP is not essential for cell survival *in vitro* [15]. The intracellular localization of TCTP is predominantly in the cytosol and nucleus [16] although it functions as an antiapoptotic protein in mitochondria. TCTP is a hydrophilic protein and does not contain any hydrophobic transmembrane domains or any localization signals to an organelle [6]. Translocation of TCTP to the nucleus under certain conditions such as oxidative stress was reported recently. However, TCTP does not contain a nuclear localization signal and the mechanism of translocation remains to be solved [17].

## 3. TCTP Interacts with Many Kinds of Proteins

To exert various physiological functions, TCTP interacts with many other proteins, including translation elongation factors eEF1A and eEF-B-$\beta$ [18], tubulin [19], actin [20], myeloid cell leukemia protein-1 (MCL1) [6, 16], Bcl-xL [21], p53 [22], and Na, K-ATPase [12]. TCTP can also bind to itself, forming homodimers [11], and this binding is required for the cytokine-like activity of this protein during allergic responses [23]. However, it is not known whether the dimerization of TCTP is necessary for its other functions.

## 4. How Does TCTP Protect Cells from Death?

It is well known that TCTP protects cells from death. Although many mechanisms have been proposed, details remain to be identified.

*4.1. TCTP Could Directly Reduce Cellular Stress.* TCTP expression increases in response to a variety of cell stresses and stimuli, and in some cases, TCTP could directly reduce stress, protecting cells from death (Figure 1). The first case we describe deals with its protection of cells from heat shock-induced cell death. TCTP is markedly upregulated in a variety of cells following thermal shock. Recent studies demonstrated that TCTP is a heat shock protein and serves as a molecular chaperone. TCTP binds to denatured proteins, refolds them, and also interacts with native proteins and protects them from denaturation [24]. Although no strong homology with other proteins has been found, recent studies revealed relationships with guanine nucleotide-free chaperones, the Mss4/Dss4 family of proteins that binds to the GDP/GTP-free form of Rab [25]. This fact also supports TCTP's function as a chaperone.

The second case is cell death induced by an influx of $Ca^{2+}$. The level of TCTP is controlled by the intracellular $Ca^{2+}$ concentration and elevation of $Ca^{2+}$ also induces TCTP mRNA in cells [26]. Binding of TCTP to $Ca^{2+}$ was demonstrated for the first time using *Trypanosoma brucei* protein [27] and later with the human protein [28]. Thapsigargin raises cytosolic $Ca^{2+}$ by blocking the ability of the cells to pump calcium into the ER, which depletes its $Ca^{2+}$ stores. This depletion can secondarily activate plasma membrane calcium channels, allowing an influx of $Ca^{2+}$ into the cytosol, thereby initiating apoptosis. The lack of TCTP resulted in exaggerated elevation of $Ca^{2+}$ in thapsigargin-challenged cells [29]. Elevation of the intracellular $Ca^{2+}$ level beyond the normal range could injure mitochondrial membranes and lead to release of cytochrome C and AIF, resulting in apoptosis. Graidist's group also demonstrated that $Ca^{2+}$ binding of TCTP is required for protection of the cells against thapsigargin-induced apoptosis. They hypothesized that TCTP exerts its antiapoptotic function by serving as a $Ca^{2+}$ scavenger. On the other hand, thapsigargin is also known to induce ER stress, in which unfolded proteins are accumulated in the organelle. Thapsigargin reduces $Ca^{2+}$ concentration in the ER and suppresses small molecule $Ca^{2+}$-dependent chaperones in the organelle, allowing accumulation of aberrant proteins, which in turn eventually leads cells to undergo apoptosis. Thus, TCTP might also protect cells from ER stress-induced apoptosis by inhibiting the signal pathway.

The last case is oxidative stress. TCTP from the parasite *Brugia malayi* has antioxidant functions and when it was overexpressed in *Escherichia coli*, it protected the cells from hydrogen peroxide-induced cell death [30]. Although TCTP used in this experiment originated in the parasite and was expressed in bacteria, this result suggests that TCTP itself might serve as antioxidant and could neutralize ROS in mammalian cells.

*4.2. TCTP Inhibits Apoptosis.* Many types of cellular stresses induce apoptosis via the mitochondrial pathway and TCTP is able to inhibit this type of apoptosis by regulating the relevant signal pathways (Figure 1). TCTP protects cells from apoptosis triggered by serum deprivation [6], or treatment with etoposide, taxol, or 5-fluorouracil [21, 31]. Mitochondria contain proapoptotic proteins such as apoptosis inducing factor (AIF), Smac/DIABLO, and cytochrome C. In the course of apoptosis, these proteins are released from mitochondria following the formation of the permeability transition pore in the membrane by the action of proapoptotic Bcl-2 family proteins such as Bax and BH3. Other Bcl-2 family members such as Bcl-2, MCL1, and Bcl-xL are known to suppress apoptosis by binding and inactivating the proapoptotic proteins. Among Bcl-2 family proteins, MCL1 is a unique protein. Unlike other Bcl-2 family proteins, MCL1 is not constitutively expressed and is induced by various stimuli. It was demonstrated that TCTP specifically associates with MCL1 [8, 16], which has the ability to stabilize TCTP [16]. In contrast to this result, another research group showed that TCTP stabilized MCL1 by suppressing its degradation by blocking its ubiquitination [8]. In their experimental conditions, MCL1 did not stabilize TCTP. The discrepancies of the results obtained from the two research groups are presumably due to the use of different cell lines and experimental conditions. TCTP and MCL1 are also capable of functioning as antiapoptotic proteins independently of each other [31]. Bcl-xL is another antiapoptotic Bcl-2 family protein that interacts with TCTP. In this case, binding between the BH3 domain of Bcl-xL and the N-terminal region of TCTP is required for

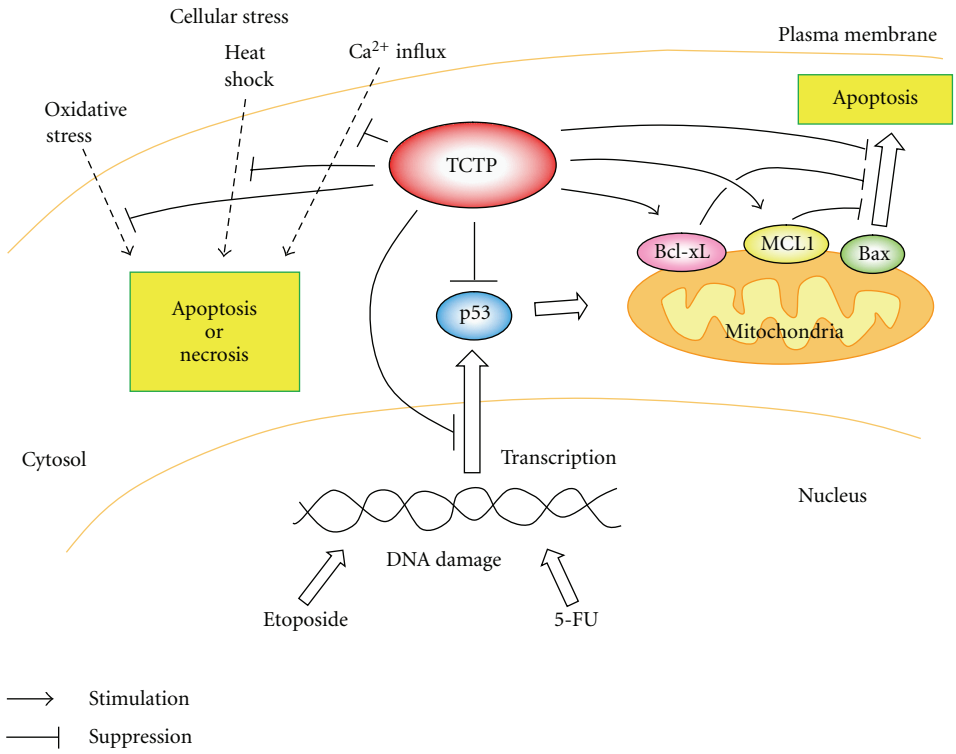


Figure 1: TCTP protects cells from cell death. TCTP inhibits cell death induced by oxidative stress, heat shock, or influx of $Ca^{2+}$. In addition, TCTP can protect cells from apoptosis triggered by treatment with genotoxic reagent such as etoposide and 5-fluorouracil. TCTP inhibits apoptosis by stabilizing antiapoptotic Bcl-2 family proteins, MCL1 and Bcl-xL and by inhibiting activation of proapoptotic Bcl-2 family protein, Bax. Moreover, TCTP inhibits p53-dependent apoptosis by downregulating the protein.

the antiapoptotic activity of TCTP [8]. The BH3 domain is responsible for hetero- and homodimerization between antiapoptotic and proapoptotic Bcl-2 family proteins. TCTP also interferes with dimerization of the proapoptotic Bcl-2 family protein Bax [32]. The crystal structure of TCTP was solved and a structural similarity with that of Bax [32] was found despite lack of amino acid sequence homology. This similarity suggests localization of TCTP to mitochondrial membranes. Dimerization of Bax is required for its apoptotic activity and TCTP blocks the formation of Bax homodimers by inserting into mitochondrial membranes (reviewed in [33]). Although TCTP inhibits apoptosis induced by Bax, unlike MCL1 and Bcl-xL, TCTP does not bind Bax directly.

TCTP affects the tumor suppressor p53 (Figures 1 and 2). The mutation in p53 is found in about half of all cancers and dysfunction of the protein is one of the main causes of cancer development. p53 is also a potent mediator of cellular responses against various cellular stresses including genotoxic insults. In addition, overexpression of p53 induces apoptosis in cancer cells. TCTP was shown to bind p53 and prevent apoptosis by destabilizing the protein in a human lung carcinoma cell line A549 [22]. TCTP also represses transcription of p53 [34]. These facts also indicate the ability of TCTP to promote transformation by reducing p53 function.

*4.3. Oxidative Stress-Induced Cell Death and TCTP.* Intrinsic reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, and hydroxyl radicals are generated in cells in the course of normal metabolism, including electron transport and various oxidase reactions. Oxidative stress induced by ROS has been implicated in aging and in the pathophysiology of various diseases such as diabetes, cancer, and Parkinson's disease (reviewed in [35]). These diseases are, at least in part, caused by ROS-mediated cell death in tissues. Although the effect of TCTP on apoptosis first attracted attention, TCTP might regulate other types of cell death. The types of cell death induced by oxidative stress depend on the cell lines and experimental conditions. In most cases, however, cell death caused by oxidative stress leads to necrosis rather than apoptosis. Types of hydrogen peroxide-induced cell death differ depending on cell types, and conditions of hydrogen peroxide treatment and cell culture. High concentrations of hydrogen peroxide inhibited apoptosis in T-lymphoma Jurkat cells by lowering intracellular ATP levels (necessary for apoptosome formation), and this might also be the case in other cell lines [36]. In the course of isolating cDNAs which protect cells from hydrogen peroxide, we found for the first time that TCTP could inhibit cell death induced by oxidative stress [37]. Overexpression of TCTP protected hydrogen peroxide-induced cell death in a Chinese hamster ovary cell line, CHO-K1; however, cell death was not typical apoptosis. Although the cells showed apoptosis-like morphological changes after hydrogen peroxide treatment, their genomic DNA did not show DNA ladder pattern formation [37]. Presumably the cells stopped apoptotic signaling after cytochrome C release from mitochondria and

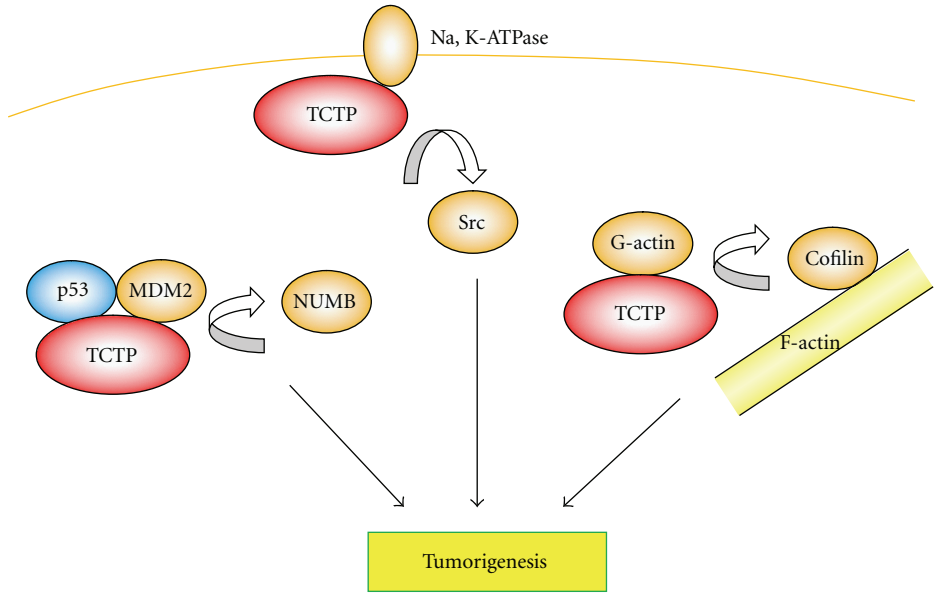


Figure 2: TCTP functions as an oncogene. NUMB forms a tricomplex with p53 and the E3 ubiquitin ligase MDM2, thereby preventing ubiquitination followed by degradation of p53. TCTP promotes p53 degradation by competing with NUMB for MDM2 binding. TCTP binds to Na, K-ATPase and, as a result, it releases Src binding to Na, K-ATPase and activate it. TCTP can release cofilin binding to G-actin by competing with and replacing cofilin. The increase of free cofilin then promotes the binding of the protein to F-actin and exerts its functions.

were subjected to secondary necrosis. Recent studies showed the existence of programmed necrosis (necroptosis) which is physiological cell death regulated by its signal pathway (reviewed in [38]). TCTP might inhibit the signal pathways of physiologically regulated necrosis. As mentioned in the previous section, it is also possible that TCTP itself acts as an antioxidant and reduces oxidative stress induced by hydrogen peroxide. This protective effect of TCTP against oxidative stress is presumably an intrinsic function in malignant breast cancer cells. Treatment with hydrogen peroxide upregulated TCTP level in T4-2 malignant breast cancer cells, but not in their parental S-1 cells that are nonmalignant [39]. TCTP upregulation was also observed in another breast cancer cell line, MDB-MB-231 after treatment with hydrogen peroxide or arsenic trioxide [40], which leads to ROS generation.

In conclusion, oxidative stress upregulates cellular TCTP levels leading to cellular protection against death. However, hydrogen peroxide treatment did not upregulate TCTP in another tumorigenic cell line (CHO-K1), although overexpression of TCTP protects cells from hydrogen peroxide [37]. The mechanisms by which oxidative stress upregulates TCTP are not known. Interestingly, TCTP translocates from cytosol to the nucleus in a keratinocyte cell line (HaCat) where it binds the vitamin D3 receptor [17]. Thus far, the physiological meaning of TCTP binding to the vitamin D3 receptor is not clear. However, this interesting phenomenon suggests that TCTP might regulate transcription of genes in response to oxidative stress. As the upregulation mediated by hydrogen peroxide is restricted to malignant cancer cells, protein factors controlling its expression could be suitable targets for cancer drug discovery. Primary culture cells of mouse embryonic fibroblasts (MEF) from TCTP knockout and control mice manifested similar proliferative activities and apoptotic sensitivities to various stimuli including hydrogen peroxide treatment [15]. These results suggest that prevention of cell death by TCTP is restricted to certain cell types such as transformed cancer cells. This hypothesis was supported by the fact that the depletion of TCTP by siRNA induced apoptosis via caspases 8 and 3 in human prostate cancer cell line LNCaP [41]. Interestingly, Mmi1P, a yeast ortholog of mammalian TCTP that binds microtubules, translocates from the cytosol to mitochondria following mild oxidative stress stimuli. In contrast to its mammalian counterpart, Mmi P has an apoptotic function in yeast cells [42].

## 5. Tumorigenicity and TCTP

Several lines of evidence indicate that TCTP can induce oncogenic transformation. Transformation of normal cells into tumor cells requires a series of genetic changes. Since TCTP is overexpressed in many types of cancer cells and silencing of the gene decreases the viability of the cells [6], it was postulated that TCTP functions as an oncogene. Tuynder et al. developed unique systems to select cells with a reverted phenotype using H-1 parvovirus which preferentially kills tumor cells [19, 43]. TCTP was found to be downregulated in reverted cells with a normal phenotype. In addition, silencing of TCTP with antisense DNA or siRNA revealed a reverted tumor phenotype, supporting this idea [19, 43, 44]. These results suggest that TCTP is directly involved in malignant transformation. Although the mechanisms of TCTP-dependent transformation are not known, it could be the result of p53 destabilization as noted in the previous section. Another line of evidence also indicates regulation of p53 by TCTP and *vice versa*. NUMB is a protein known to be a regulator of p53. It forms a tricomplex with p53

and the E3 ubiquitin ligase MDM2, thereby preventing ubiquitination followed by degradation of p53 [45]. TCTP promotes p53 degradation by competing with NUMB for MDM2 binding (Figure 2). On the other hand, p53 directly represses transcription of TCTP. Thus, TCTP and p53 form a reciprocal negative regulation loop [34]. This fact also suggests that TCTP might inhibit p53-dependent apoptosis by downregulating the protein.

The most important properties of tumor and cancer cells are unregulated cell proliferation and avoidance of cell death. Inhibition or gene silencing by TCTP siRNA reduces viability and induces apoptosis in cancer cells, including human prostate cancer cells [41]. TCTP might also be involved in the malignancy of tumors by interacting with actin at the cofilin binding site. Cofilin is an actin binding protein and has the ability to regulate the cell cycle (reviewed in [46]) and promote metastasis [47]. TCTP competes with cofilin at the cofilin-binding site of actin. Although cofilin can bind to both monomeric (G-actin) and filamentous actin (F-actin), it exerts its functions by binding to and changing the twist of F-actin. On the other hand, TCTP has a higher affinity with G-actin than F-actin. TCTP can release cofilin binding to G-actin by competing with and replacing cofilin. The increase of free cofilin then promotes the binding of the protein to F-actin and exerts its functions (Figure 2) [20]. Recent studies also revealed that TCTP induces transformation in human breast epithelial cells through activation of a protooncogene product Src [48]. TCTP binds to the $\alpha$1 subunit of Na, K-ATPase and, as a result, it releases Src binding to Na, K-ATPase. This TCTP-mediated Src release activates Src and promotes various tumor progression signal pathways (Figure 2) [48].

## 6. TCTP Regulates Cell Proliferation

Since TCTP is highly expressed in actively dividing cells [28, 49], one might expect TCTP to modulate physiological functions during cell proliferation. TCTP has the ability to bind microtubules during G1-, S-, G2-, and M-phases of the cell cycle. It associates with the metaphase spindle, but is detached from the spindle after metaphase [50]. TCTP is phosphorylated by the polo-like kinase Plk, which is likely to cause detachment of TCTP from the mitotic spindle [9]. Since the TCTP level is upregulated during entry into the cell cycle, the protein is believed to be important for cell growth and division. TCTP overexpression in mammalian cells results in cell cycle retardation, microtubule stabilization, and alteration of cell morphology [49]. Furthermore, TCTP mutated in the phosphorylation sites for Plk disrupts the completion of mitosis, indicating the importance of TCTP phosphorylation in normal cell cycle regulation [9]. The fact that increased TCTP levels slow cell cycle progression is unexpected because high levels of TCTP expression are generally observed in actively dividing cells and the discrepancy has yet to be explained.

TCTP might regulate proliferation through the target of rapamycin (TOR) pathway. TOR is a Ser/Thr kinase that regulates proliferation and metabolism in response to nutrients, hormones, and growth factors. In case of *Drosophila*, *Dorsophila* TCTP (dTCTP) binds to nucleotide free form of a small GTPase, *Drosophila* Ras homolog enriched in brain (dRheb), and stimulates GDP-GTP exchange of dRheb. As a result, dTCTP activates the TOR signaling pathways. In fact, tissue-specific reduction of dTCTP *in vivo* resulted in smaller organs with reduction of both cell size and cell number [51]. This might be also the case in mammals [52].

## 7. Concluding Remarks

We have reviewed recent findings on biological effects of mammalian TCTP, focusing on inhibition of cell death, regulation of proliferation, and tumorigenesis. Although many hypotheses have been proposed, mechanistic explanations of TCTP on phenomena are still elusive. Presumably, TCTP is able to modulate multiple protein targets simultaneously and as a result, it exerts effects. Further comprehensive studies are necessary to clarify the detailed mechanisms. Recent studies also suggest protective functions of TCTP against cell death other than apoptosis. The mechanisms of TCTP's action on the cell death is interesting and important issues in future studies.

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*Research Article*

# Cytoplasmic and Nuclear Localization of TCTP in Normal and Cancer Cells

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*Objective*. Intracellular localization of translationally controlled tumour protein (TCTP) was investigated in cancer cells. *Methods*. The expression and localization of TCTP were detected at 12 h, 24 h, 48 h, 60 h time points in culture of human hepatocarcinoma cell line HepG2, human cervical carcinoma cell line HeLa, and human normal liver cell line HL-7702 by immunofluorescence. *Results*. TCTP was expressed in both normal and tumor cells, and its localization changes at different time points. TCTP was mainly expressed in cytoplasm from 24 h to 48 h then expressed in both nucleus and cytoplasm at 60 h in HL-7702 cells. While in HepG2 cells, TCTP first localized at cell membrane within 24 h then at both nucleus and cytoplasm from 48 h to 60 h; TCTP localized at both nucleus and cytoplasm from 12 h to 60 h in Hela cells. *Conclusion*. The translocation of intracellular expression of TCTP in normal and tumor cells at different time points may pave a path to the studying of TCTP role in tumor growth.

## 1. Background

The translationally controlled tumor protein (TCTP), a highly conversed protein [1, 2], also called the histamine releasing factor (HRF) [3], has been suggested as a tumor-associated antigen and widely expressed in mammals as well as in a wide range of other organisms of both animal and plant kingdom [4]. Its mRNA and protein expression levels tend to be higher in the colorectal cancers (CRCs) [5] and hepatocellular carcinoma [6], compared to the corresponding normal tissues. TCTP was found to be the most strikingly downregulated in tumor reversion [7]. Moreover, the level of TCTP in the revertants from three other major solid cancers, colon, lung, and melanoma cell lines, has the same results [5, 8, 9]. In addition, transfection with antisence of TCTP attenuated malignancy of v-src-transformed NIH3T3 cells. Recently, TCTP has attracted the attention of an increasing number of researchers interested in various biologically and medically relevant processes. This is largely due to the fact that TCTP levels are highly upregulated in response to a wide range of extracellular stimuli [10, 11]. A series of recent reports highlighted the importance of TCTP for cell cycle progression and malignant transformation [12, 13]. TCTP was also shown to display an extracellular function as a histamine release factor and to have antiapoptotic activity [14].

The intracellular localization of TCTP remains controversy. It was shown to localize in cytoplasm by Arcuri et al. [15] and nucleus by Li et al. [14], respectively. TCTP can be secreted and tumor-suppressor-activated pathway-6 (TSAP6) facilitates the secretion of TCTP via a nonclassical pathway through exosomes which highlights the association of TCTP and TSAP6 in cytoplasm [16]. The purpose of our study was to determine the localization of TCTP expression in two human cancer cell lines and one normal cell line. We applied cell immunofluorescence and detected the protein expression of TCTP at different time points in these three cell lines. We found that TCTP localized in both cytoplasm and nucleus and its translocation varied between normal and tumor cell lines at different time points.

## 2. Materials and Methods

*2.1. Cell Culture.* Human hepatocellular carcinoma cell line HepG2, human cervical cancer cell line HELA, and

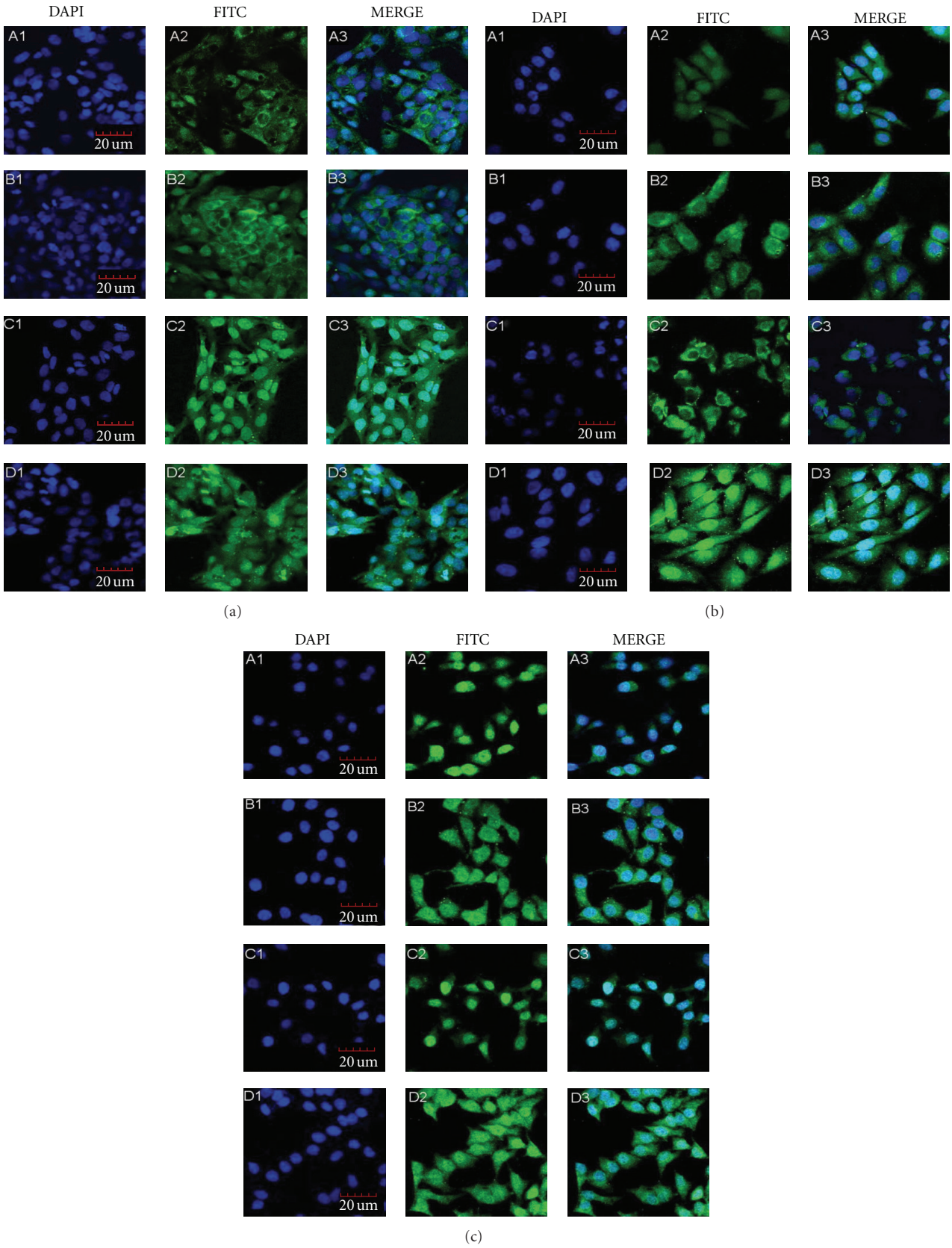


Figure 1: Localization of TCTP in HepG2 cells (a), HL-7702 cells (b) and HeLa cells (c), A1–A3: 12 h, ×400; B1–B3: 24 h, ×400; C1–C3: 48 h, ×400; D1–D3: 60 h, ×400.

human normal hepatocyte cell line HL-7702 were purchased from Chinese Academy of Science (Shanghai) and grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO, Invitrogen, USA) supplemented with 10% FBS (ExCell, Genetimes, China), maintained at 37°C, 95% humidity, and 5% carbon dioxide.

*2.2. Reagent and Antibodies.* The rabbit polyclonal antibody for TCTP, rabbit polyclonal antibody for TSAP6, and goat polyclonal secondary antibody to rabbit IgG-H & L (FITC) were purchased from Abcam. Paraformaldehyde and Tween20 were purchased from Sigma-Aldrich. The 4,6-Diamidine-2-phenylindole dihydrochloride (DAPI), Bovine Serum Albumin (BSA), antifade mounting medium, and phosphate-buffered saline were purchased from Roche, Solarbio, Beyotime, and Zhongshan Golden Bridge Biotech, respectively.

*2.3. Immunostaining and Microscopy.* For indirect immunofluorescence, HepG2, HL-7702, and HeLa cells were cultured on coverslips and fixed with 4% paraformaldehyde after 12 h, 24 h, 48 h, and 60 h culture, respectively, then permeabilized with 0.2% Tween 20, blocked with 1% BSA for 1 h, and incubated with the rabbit polyclonal to TCTP (1 : 500) and TSAP6 (1 : 200), respectively, overnight at 4°C, coupled with the secondary antibody at 4°C for 6 hours. DAPI was used to stain the nucleus. Immunofluorescence staining imaging was captured using Laser Confocal Scanning Microscope (LCSM).

## 3. Results

*3.1. Localization of TCTP.* As shown in Figure 1, TCTP protein was mainly localized in the cytoplasm and membrane from 12 h to 24 h, then in both nucleus and cytoplasm from 48 to 60 h in HepG2 cells (Figure 1(a)). In HL-7702 cells, the localization of TCTP protein showed a "nucleus-cytoplasm-nucleus" pattern at different time points (Figure 1(b)). TCTP localized in nucleus and cytoplasm in HeLa cells at every time point (Figure 1(c)).

## 4. Discussion and Conclusion

Previous reports from Arcuri's group [15] have demonstrated localization of TCTP protein in human prostate and prostate cancer cells using immunohistochemistry and immunofluorescence staining. The protein was mainly expressed in the secretory luminal epithelial and basal layer cells. A significant amount of protein was present in the prostatic fluids. Subcellular distribution studies on prostate epithelial cells showed the protein localized in the cytoplasm in interphase and colocalized with tubulin during mitosis. Li's group [14] has demonstrated that the intracellular localization of TCTP was present predominantly in the nucleus in HeLa cells after transfection with exogenous TCTP expression plasmid. Amson et al. and Ohgami et al. found that TCTP was secreted through an endoplasmic reticulum/Golgi-independent or nonclassical pathway and that the secreted TCTP was originated from preexisting pools. TSAP6, a p53-inducible 5-6 transmembrane protein [17, 18], was found to interact with TCTP and they partially colocalized in exosomes, some vesicular-like structures at the plasma membrane and around the nucleus. Functionally, the overexpression of TSAP6 consistently leads to enhanced secretion of both endogenously and exogenously expressed TCTP [16, 17]. We initiated our work by asking whether TCTP expression can be translocated and what is the relationship between its secretion level and function. For the first time, using the approach of immunofluorescence staining, we found that TCTP localized at different part within cells via several experiments. As demonstrated above, TCTP protein expression could be translocated along with cell growth.

Interesting phenomena of our results were that TCTP localization differed between cell lines at the same time points and that TCTP did not change its cellular localization in HeLa cells, which can be explained as follows. (1) Different characteristics of cell lines. Firstly, HeLa cell is immortal because of being infected by the human papilloma virus (HPV). Unlike HeLa, HepG2 cells and HL-7702 cells have different cell cycles and are easy to die. Secondly, different cell lines have different cell cycles in which TCTP plays a key role. (2) Different TCTP expression levels. TCTP is an antiapoptotic and conserved protein. Numerous studies have shown that TCTP expression level in tumor is higher than that in the corresponding normal tissues, and inhibition of TCTP expression can attenuate malignant phenotypes [7], indicating TCTP has a critical role in tumorigenesis. Therefore, translocation of subcellular TCTP at different times might reflect its biological functions, and the underlying mechanism needs further investigation.

In summary, we reported the localization and dynamic translocation of TCTP in different cell lines. TCTP localized in both cytoplasm and nucleus and it translocated into different subcellular units along with cells growth. Our finding provides more evidence showing the association between TCTP localization and function, improving our understanding of the important role of TCTP in cancer formation.

## Authors' Contribution

Y.-P. Ma carried out the experiments and cowrote the paper; W.-L. Zhu directed the research and cowrote the paper. All authors read and approved the final paper.

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*Research Article*

# Association of TCTP with Centrosome and Microtubules

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Translationally Controlled Tumour Protein (TCTP) associates with microtubules (MT), however, the details of this association are unknown. Here we analyze the relationship of TCTP with MTs and centrosomes in *Xenopus laevis* and mammalian cells using immunofluorescence, tagged TCTP expression and immunoelectron microscopy. We show that TCTP associates both with MTs and centrosomes at spindle poles when detected by species-specific antibodies and by Myc-XlTCTP expression in *Xenopus* and mammalian cells. However, when the antibodies against XlTCTP were used in mammalian cells, TCTP was detected exclusively in the centrosomes. These results suggest that a distinct pool of TCTP may be specific for, and associate with, the centrosomes. Double labelling for TCTP and $\gamma$-tubulin with immuno-gold electron microscopy in *Xenopus laevis* oogonia shows localization of TCTP at the periphery of the $\gamma$-tubulin-containing pericentriolar material (PCM) enveloping the centriole. TCTP localizes in the close vicinity of, but not directly on the MTs in *Xenopus* ovary suggesting that this association requires unidentified linker proteins. Thus, we show for the first time: (1) the association of TCTP with centrosomes, (2) peripheral localization of TCTP in relation to the centriole and the $\gamma$-tubulin-containing PCM within the centrosome, and (3) the indirect association of TCTP with MTs.

## 1. Introduction

Translationally Controlled Tumour Protein (TCTP) is implicated in a broad diversity of cellular functions. It stimulates cell proliferation, growth, survival, and stress response [1]. It is very abundant in highly proliferating cells, including cancer cells. The interest in TCTP increased rapidly in recent years because of the growing body of evidence for its key role in carcinogenesis and rare phenomenon of tumour reversion [2, 3]. Recently, it was elegantly demonstrated that TCTP expression is negatively regulated by p53 and *vice versa*, that is, TCTP negatively regulates p53 cellular levels via induction of its degradation triggered by MDM2 ubiquitin ligase [4]. The evidence of the reciprocal feedback between TCTP and p53 gives additional proof of the importance of TCTP in cancer development and progression/reversion. TCTP is also associated with the cytoskeleton and throughout this association impacts cell shape, motility, metastasis, and the aggressiveness of cancer. It has been established that TCTP associates both with actin microfilaments (MFs) and MTs [5]. Biochemical analysis of these interactions suggested that, most likely, the TCTP interacts with MFs and MTs indirectly; however, details of these interactions remain unknown (ibid.). TCTP knock down modifies drastically the cell shape and both MFs and MTs architecture [5, 6]. TCTP acts in competition with actin-binding protein cofilin [7]. Because the cofilin promotes actin disassembly, the competition with TCTP may result in increased actin polymerization in cells

with higher TCTP levels. Much less is known about the relationship between TCTP and MTs. We have shown that TCTP and tubulin localization in *Xenopus* and human cells are very similar, but not identical suggesting a presence of "TCTP fibers" unrelated to MTs as well as the presence of TCTP-negative MTs [5]. TCTP localization within the mitotic spindle also does not overlap tubulin localization—it has more homogenous pattern, which suggests that either only a subpopulation of TCTP is associated with MTs or that TCTP localizes in the vicinity but not directly on MTs. On the other hand, TCTP seems to be very strongly associated with the poles of the spindle [5]. These observations suggested that TCTP may be associated with MTs via intermediate linker proteins and that TCTP may also be centrosome-associated protein. We investigated these hypotheses in the study presented here.

## 2. Material and Methods

*2.1. Tissue Culture Cells.* The XL2 cell line was cultured in L-15 medium supplemented with 10% fetal calf serum (FCS; full medium) and incubated at 25°C in air. HeLa, NIH3T3, and Cos7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS) and incubated at 37°C in 5% $CO_2$. Media were supplemented with penicillin (100 Units/mL) and streptomycin (100 mg/mL).

*2.2. Immunocytochemistry.* Cells seeded on glass coverslips were fixed in 75% methanol, 3.7% formaldehyde, 0.5x PBS, or in 3.7% paraformaldehyde in 1x PBS for 10 min at room temperature and permeabilized with 0.1% Triton X100 in PBS for 5 min. DNA was visualized using DAPI. Polyclonal antibodies against XlTCTP (produced in the laboratory in Rennes) and against HsTCTP (Santa-Cruz) or rat TCTP were used at the dilution of 1 : 1000 and 1 : 100, respectively, with overnight incubations at 4°C. Anti-$\alpha$-tubulin (Sigma) and anti-$\beta$-tubulin (Euromedex) were diluted 1 : 200. Purified anti-c-myc antibody (Sigma) was diluted 1 : 100. Secondary antibodies (RITC conjugated, 1 : 1000 dilution; Molecular Probes) were incubated for 1 hr at room temperature. Coverslips were mounted in Vectashield and examined using a Leica DMRXA2 fluorescence microscope or Leica Confocal SP2 microscope. Photographs were taken using a black and white COOLsnap ES camera (Roper Scientific), and images were processed using Metamorph software (Universal Imaging).

*2.3. Cell-Free Extracts and In Vitro Spindle Assembly.* Cytostatic factor-arrested extracts (CSF extracts) were prepared as described by Murray [8]. For *in vitro* spindle assembly, 0.5 $\mu$L of rhodamine-labeled bovine brain tubulin (Cytoskeleton) was added at 0.2 mg/mL and 2 $\mu$L of sperm heads at a concentration of ~1000 nuclei/$\mu$L added to 50 $\mu$L of the extract and incubated for 60–90 min at 21°C. Spindles (15 $\mu$L of extract) were prefixed in 1 mL BRB80 buffer (80 mM K-Pipes, pH 6.8, 1 mM EGTA, and 1 mM $MgCl_2$) containing 30% glycerol, 1% paraformaldehyde, and 0.5% Triton X-100 and centrifuged (2300 xg, 30 min at room temperature) through a 40% glycerol cushion in BRB80 onto glass coverslips in 12-well plate. They were fixed by adding 1 mL cold methanol (−20°C) for 10 min at room temperature (isolated spindles). Then fixed spindles were processed for immunocytochemistry for TCTP using anti-XlTCTP, viewed, and photographed as the cells above.

*2.4. Cell Transfection.* For transfection of XL2 and NIH3T3 cells with plasmids encoding *Xenopus* Myc-TCTP, $5 \times 10^5$ cells were plated on glass coverslips in a 12-well plate. Cells were transfected with 0.5 $\mu$g of plasmid DNA using FuGENE 6 transfection reagent (ROCHE) following the manufacturer's instructions.

*2.5. Mouse Oocytes.* Three-month-old Swiss albino females were injected intraperitoneally with 10 IU pregnant mare serum gonadotrophin (PMSG; Folligon, Intervet, Holland) to stimulate the development of ovarian follicles. Forty eight to fifty two hours later females were killed by cervical dislocation. Fully grown oocytes arrested at prophase of the first meiotic division—germinal vesicle stage (GV) were released from ovarian follicles. Oocytes were freed from cumulus cells by pipetting and then cultured for 2 h in M2 medium containing bovine serum albumin (BSA; 4 mg/mL). Oocytes that resumed meiosis, that is, underwent germinal vesicle breakdown (GVBD) within first 2 h of *in vitro* culture, were used for further manipulations and collected for the following stages: GVBD, MI, (6 hrs after GVBD), and MII (20 hrs after GVBD). Oocytes were fixed in 3.7% formaldehyde in PBS, permeabilized with 0.01 Triton X100 in PBS, and subjected to immunofluorescence after incubation in the presence of XlTCTP antibody, the same as with the tissue culture cells.

*2.6. Xenopus Laevis Tadpole Ovaries and Electron Microscopy.* The developing ovaries were removed from anaesthetized tailed and tailless froglets (stages 62–66) of wild-type *Xenopus laevis*. Ovaries were fixed in TEM fixative (2% formaldehyde, 3% glutaraldehyde, EM grade, Ted Pella, Redding, CA, in 0.1 M sodium cacodylate buffer pH 7.3, Polysciences, Warrington, PA) containing 10 $\mu$m taxol (Cytoskeleton, Denver, CO) to stabilize the microtubules and centrioles. To enhance the visualization of centrioles, the material was stained in 0.5% uranyl acetate, and the osmium tetraoxide treatment was omitted. This resulted in very light staining of all membranous structures; however, it allowed the visualization of highly contrasted centrioles and microtubules. Embedding and sectioning were done as described by Kloc et al. [9]. Postembedding immunostaining using anti-XlTCTP and anti-$\gamma$-tubulin antibodies was performed as described in Bilinski et al. [10]. For immunogold labeling, the ovaries were fixed as above. Ultrathin sections (60 nm thick) were collected on nickel single-slot grids (coated with formvar) and blocked with 2% bovine serum albumin (BSA; Sigma) in PBS and 0.1% NaN3 for 30 min, after overnight incubation at 4°C with the primary antibodies (rabbit anti-TCTP, or mouse monoclonal anti-$\gamma$-tubulin [GTU-88], ab11316, Abcam) diluted 1 : 50−1 : 100 in the incubation solution (PBS, 1% BSA, 0.1% NaN3). Following several washes in PBS, the grids were incubated for two hours, at room

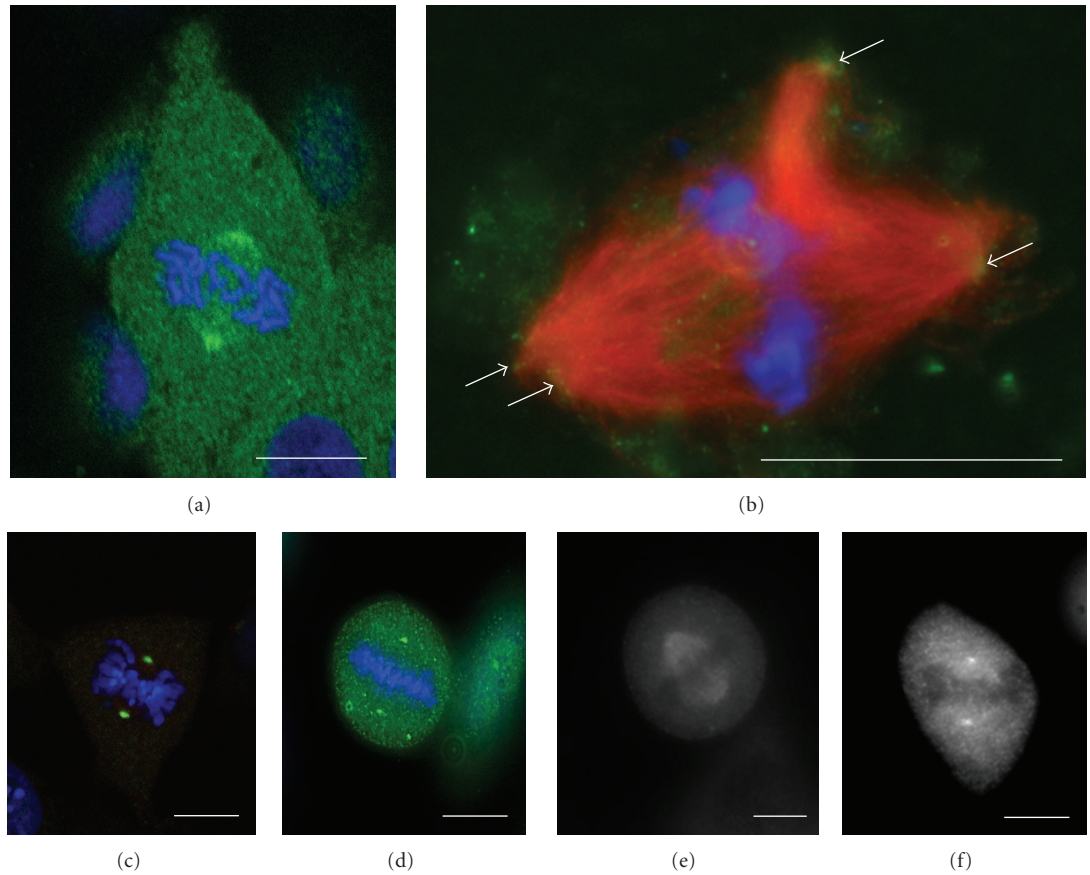


Figure 1: Immunofluorescence localization of TCTP in *Xenopus laevis* mitotic spindles using XlTCTP antibody. (a) Confocal section of an XL2 cell showing the presence of TCTP in the spindle with higher density at the spindle poles. (b) Isolated spindle formed by sperm-head addition to the CSF extract. Red MTs stained with rhodamine-$\beta$-tubulin added to the extract, green TCTP detected by immunofluorescence with XlTCTP antibody. White arrows point to spindle poles with TCTP staining. Blue DNA stained with DAPI. Note the presence of yellow staining of TCTP at the spindle poles and the absence of TCTP in the remaining parts of the spindle. (c) Confocal section of murine metaphase NIH3T3 cell stained with XlTCTP antibody (green) and with DAPI for DNA. Note that XlTCTP stains exclusively two distinct spots corresponding to the centrosomes, at the spindle poles corresponding. (d) Confocal section of human HeLa metaphase cell. Green TCTP detected with XlTCTP antibody, blue DNA. XlTCTP stains two spindle poles; the granular background staining is also visible in the cytoplasm. (e) Human HeLa metaphase cell. Green TCTP detected with homologous HsTCTP antibody, blue DNA. HsTCTP stains the whole spindle. (f) Monkey Cos7 metaphase cell incubated with anti-rat TCTP antibody showing a very distinct staining of spindle poles. Bar is equal to 20 $\mu$m.

temperature, with the secondary antibody (goat anti-rabbit conjugated to 18 nm gold particles or goat anti-mouse conjugated to 10 nm gold particles, Jackson ImmunoResearch Lab.) diluted 1 : 100–1 : 200 in the incubation solution. Subsequently, the grids were washed in PBS and finally in distilled water. After drying, the sections were contrasted with uranyl acetate and lead citrate and viewed with a JEOL 100SX electron microscope at 80 kV. In control experiments, sections were treated exactly the same as described above, but there was no incubation with the primary antibody. The secondary antibodies were also tested for cross-reactivity prior to double labeling experiments.

## 3. Results and Discussion

We focused our analysis on the localization of TCTP within the mitotic spindle because it allowed us to study simultaneously the association of TCTP with MTs and with centrosomes, which are located at the spindle poles. Immunolocalization of TCTP in mitotic *Xenopus laevis* XL2 cells clearly showed the presence of TCTP in the mitotic spindle with higher concentration at the spindle poles (Figure 1(a)). The spindle pole accumulation of TCTP was also evident in the spindles isolated from M-phase-arrested cell-free extract (Figure 1(b)). Because in mitotic *Xenopus laevis* cells and cell-free extract, the TCTP is associated with the spindle poles where the centrosomes are located, this suggests that TCTP may be a centrosomal protein.

TCTP is very evolutionary conserved protein [11]. Thus, we tested antibodies directed against different species TCTP in *Xenopus laevis* and mammalian cells. Surprisingly, when we used our polyclonal antibody against *Xenopus laevis* TCTP (XlTCTP) for TCTP detection in murine NIH3T3 and human origin HeLa cells (so called heterologous or

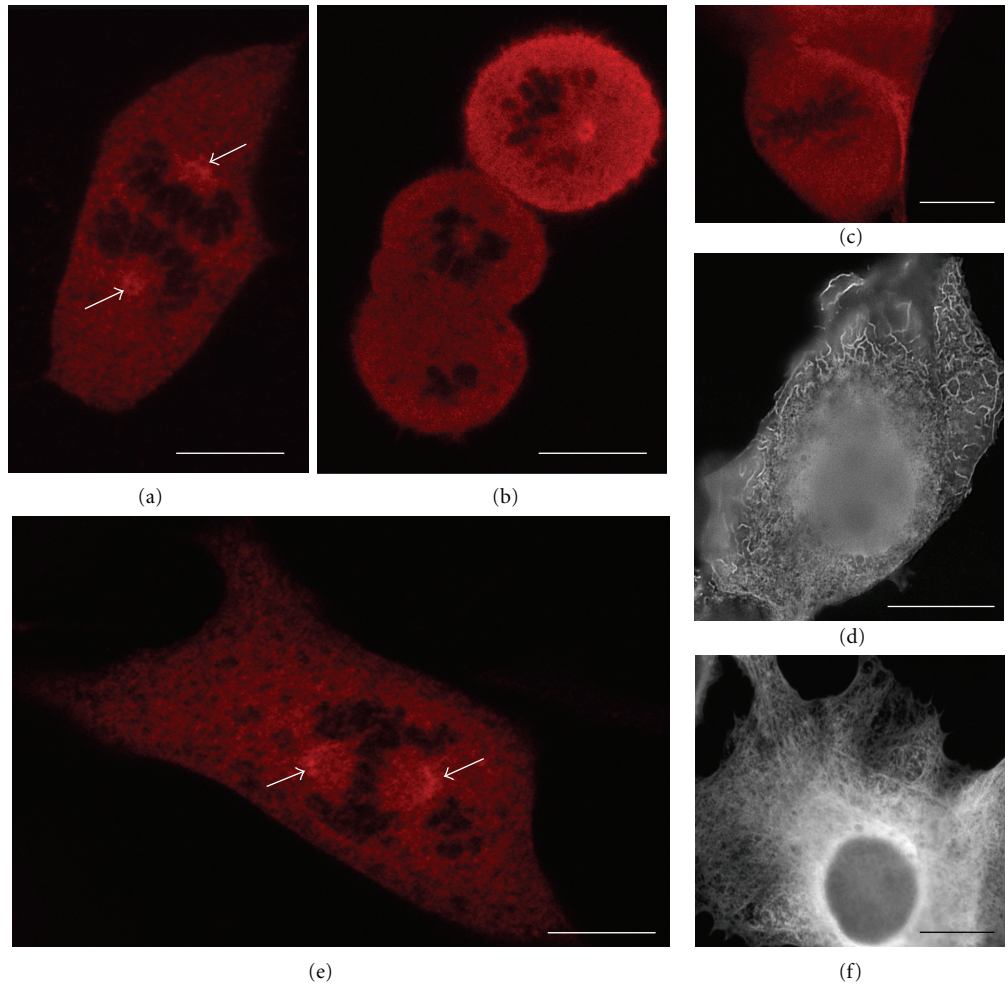


Figure 2: Expression of Myc-XlTCTP in *Xenopus laevis* XL2 cells. (a) Confocal section of XL2 cell in anaphase with high concentration of XlTCTP at the spindle poles (white arrows). (b) Confocal section of two dividing XL2 cells with high concentration of XlTCTP at the spindle poles. (c) Control mitotic XL2 cells expressing Myc tag only. (d) Interphase XL2 cell expressing Myc-XlTCTP. XLTCTP is localized in distinct fibers in the cytoplasm. (e) Mitotic murine NIHT3T cell expressing Myc-XlTCTP. High concentration of XlTCTP is present at the spindle poles (white arrows). (f) Interphase murine NIH3T3 cells expressing Myc-XlTCTP. Note that XlTCTP forms MT-like fibers in the cytoplasm. Bar is equal to 20 $\mu$m.

interspecies detection), we always observed a very bright staining of centrosomes at the spindle poles (Figures 1(c) and 1(d)). However, when we used homologous antibodies and cells, that is, anti-human TCTP antibody to detect TCTP in human HeLa cells, a uniform staining of the whole spindle was visible (Figure 1(e)), which agreed with our previous study [5] and studies by Gachet and colleagues [12]. When we used another heterologous combination, that is, the-anti-rat TCTP antibody in monkey Cos7 cells, we also detected clear centrosomal staining (Figure 1(f)). These observations suggest that the subpopulations of immunologically distinct TCTP might be present in the mitotic centrosomes of human and monkey cells, similarly as in *Xenopus laevis* cells.

To further clarify these observations, we expressed Myc-tagged XlTCTP in *Xenopus laevis* XL2 cells (homologous expression) and in mouse NIH3T3 cells (heterologous expression) and followed the localization of the recombinant frog protein in these two types of cells via immunofluorescence with anti-myc antibody. Figures 2(a) and 2(b) show examples of anti-Myc immunodetection of exogenous XlTCTP in XL2 cells. In these cells, we always observed MT-associated localization and an accumulation of Myc-tagged XlTCTP around a small negative area at the very tip of the spindle (Figures 2(a) and 2(b)). The control cells expressing Myc tag alone were uniformly stained (Figure 2(c)). In addition, in the interphase XL2 cells, the Myc-XlTCTP was incorporated into distinct cytoplasmic fibers (Figure 2(d)). The Myc-XlTCTP expression in murine NIH3T3 cells resulted in strong localization of TCTP to the spindle poles; however, we have never observed the presence of the TCTP-negative area similar to the one visible in XL2 cells (Figure 1(e)). In the interphase NIH3T3 cells expressing Myc-XlTCTP the frog TCTP was incorporated to the MT-like fibers (Figure 2(f)). These results show that

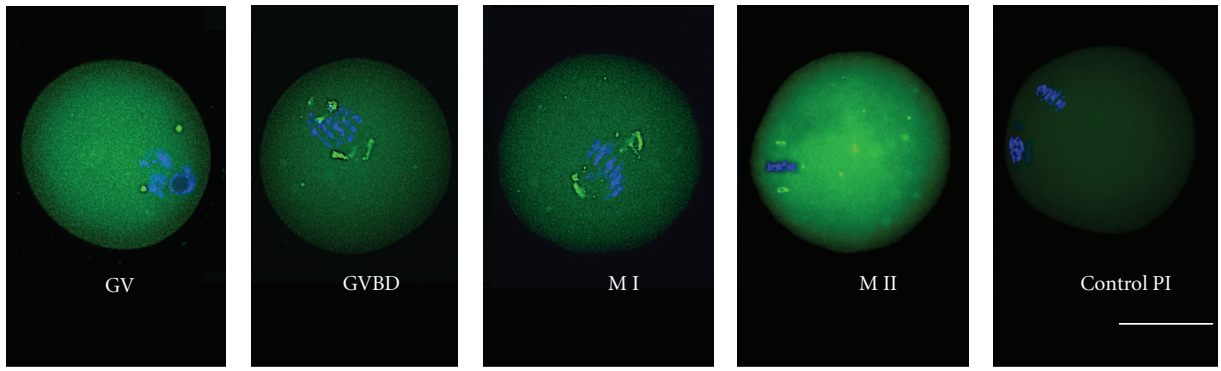


Figure 3: Immunofluorescence localization of TCTP with anti-XlTCTP antibody in mouse maturing oocytes. GV: prophase I-arrested oocyte, GVBD: the beginning of maturation, MI and MII: oocytes in MI and MII phase of meiosis, respectively, control PI: control MII oocyte stained with the preimmune serum. XlTCTP antibody stains PCM in all stages of maturing mouse oocytes. DNA (blue) stained with DAPI. Bar is equal to 40 $\mu$m.

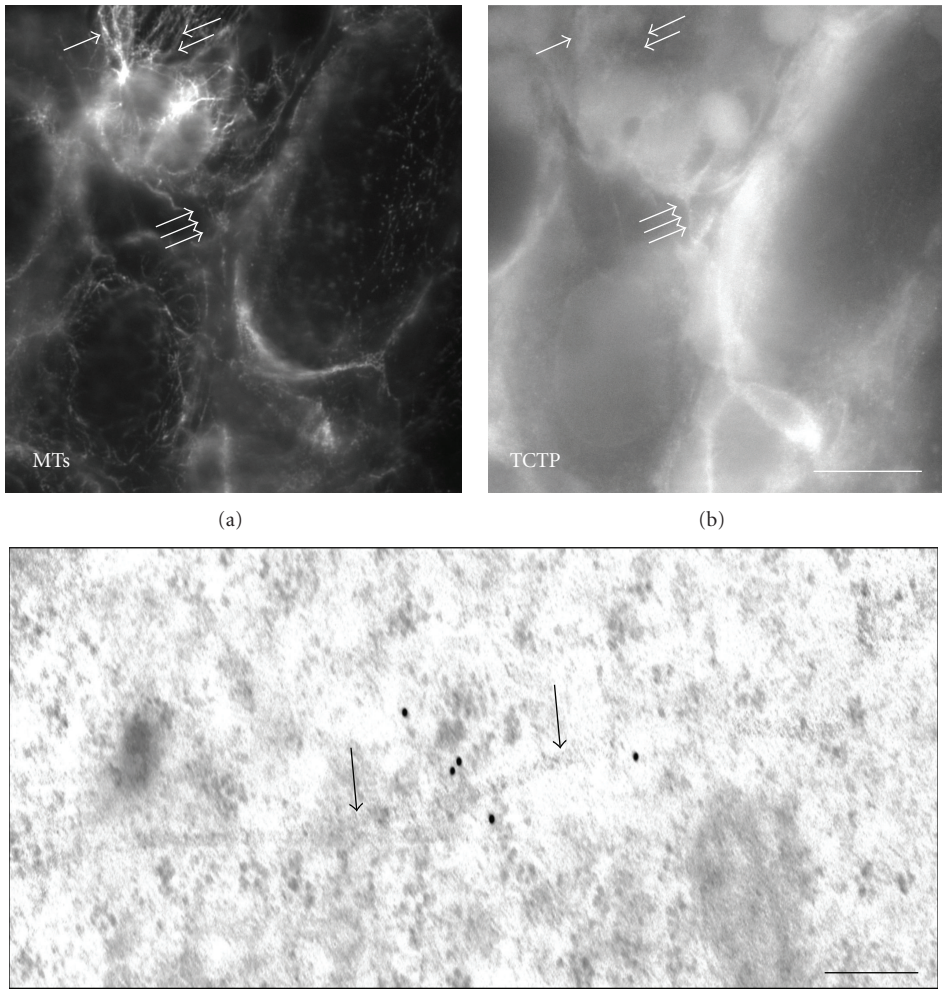


Figure 4: MTs and TCTP in *Xenopus laevis* tadpole ovary. Interphase cells. Upper panel, left: $\beta$-tubulin, right: TCTP localization. Anti-XlTCTP was used for this localization. Single white arrow points to cellular structures positive both for $\beta$-tubulin and TCTP. Double arrows point to $\beta$-tubulin-positive and TCTP-negative fibers. Triple arrows point to TCTP-positive and $\beta$-tubulin-negative fibers. Bar is equal to 20 $\mu$m; Bottom panel: electron microscopy gold immunolabeling of TCTP (black particles in the center) in the vicinity of MTs (black arrows). Bar is equal to 100 nm.

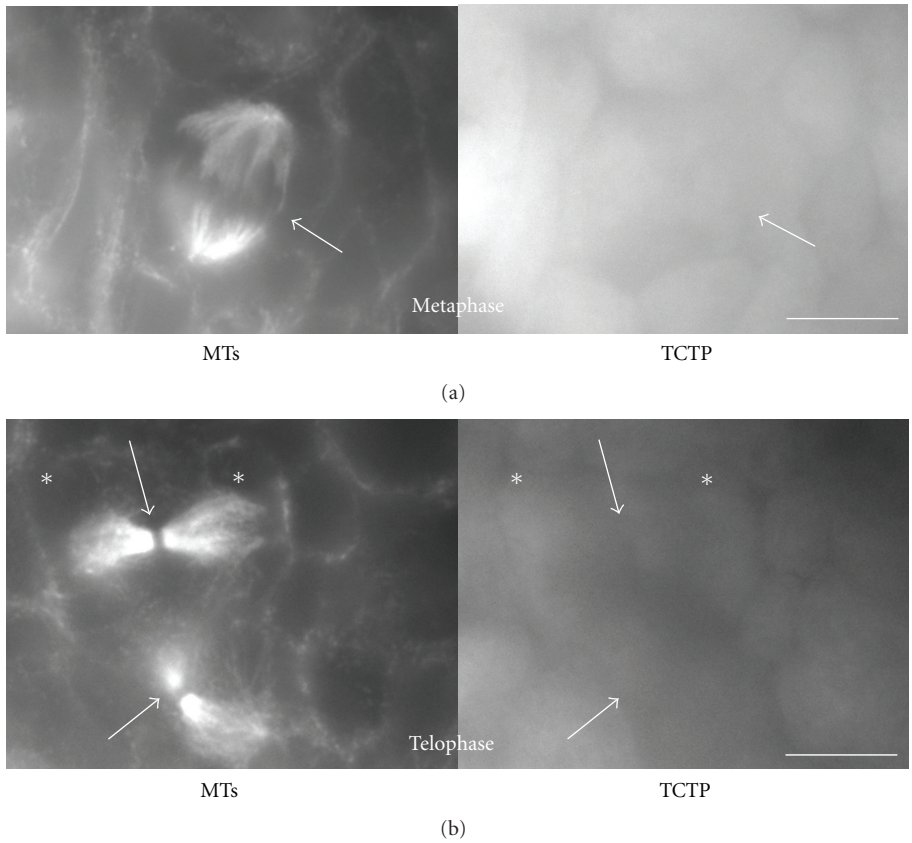


Figure 5: MTs and TCTP in mitotic *Xenopus laevis* oogonia. Left: $\beta$-tubulin, right: TCTP localization in tadpole oogonia. Anti-XlTCTP was used for this localization. Upper panel: metaphase cell. Left: mitotic spindle visualized by $\beta$-tubulin staining (white arrow). Right: The whole of the spindle is positive for TCTP (white arrow). Bottom panel: two telophase oogonia. Left: prominent midbides are visualized by anti-$\beta$-tubulin immunoflorescence (white arrows). White asterisks show the position of two daughter cells. Note the absence of TCTP in the midbodies. Bar is equal to 20 $\mu$m.

TCTP indeed localizes to the spindle poles both in *Xenopus laevis* and in mouse cells, but the pattern of its localization is slightly different when homologous and heterologous system of immunodetection is used. Thus, exogenous Myc-XlTCTP is incorporated to the pericentrosomal area in the mitotic XL2 cells, while in the mitotic mouse cells, it is incorporated into the whole mitotic centrosomes. On the other hand, the homogenous immunofluorescence staining of XlTCTP visible in the spindle poles of XL2 cells suggests the presence of XlTCTP within the whole centrosomes. This indicates that, depending on the species or the cell type, the TCTP is localized either at the spindle pole within the centrosome or around the centrosome in the pericentriolar material (PCM) composed of specific proteins (including $\gamma$-tubulin).

Mouse oocytes have no centrioles [13, 14], but they have irregular foci of PCM at the spindle poles both in MI and MII phases of meiosis [15–17]. Because mouse oocyte have PCM but do not have centrioles, we used maturing mouse oocytes to analyze whether TCTP associates with the PCM foci. When we stained *in vitro* maturing mouse oocytes with the anti-XlTCTP, we detected typical images of PCM foci (Figure 3) instead of the whole spindle staining observed when anti-rabbit TCTP antibody was used on mouse oocytes [18]. In GV stage oocytes arrested in prophase of the first meiotic division, a few distinct foci may be detected which are localized mainly next to the oocyte nucleus (called GV for Germinal Vesicle; Figure 3(a)), thus showing the number and pattern of distribution typical to PCM [19]. After GVBD (germinal vesicle breakdown) and during MI and MII, the TCTP-positive foci polarize at the relatively broad spindle poles (Figures 3(b), 3(c), and 3(d)). The same polarization of the PCM foci was shown by Schatten et al. [16], and Maro et al. [17]. Taken together, these results indicate that the subpopulation of TCTP detected by anti-Xenopus TCTP antibody indeed localizes to the PCM foci.

In contrast to mouse oocytes, *Xenopus laevis* oogonia (or nest cells) have typical centrosomes formed by centrioles and the PCM [9]. We used these cells to analyze TCTP localization in relation to the MTs and centrosomes using light microscopy immunofluorescence and immunogold electron microscopy detection. Immunofluorescence using anti-$\beta$-tubulin and anti-TCTP antibodies in nest cells showed that the distribution of these two proteins was similar to their distribution in XL2 cells, that is, in the majority of cases these two proteins colocalized, but a subpopulation of MTs devoid of TCTP was also detected, and some TCTP-rich

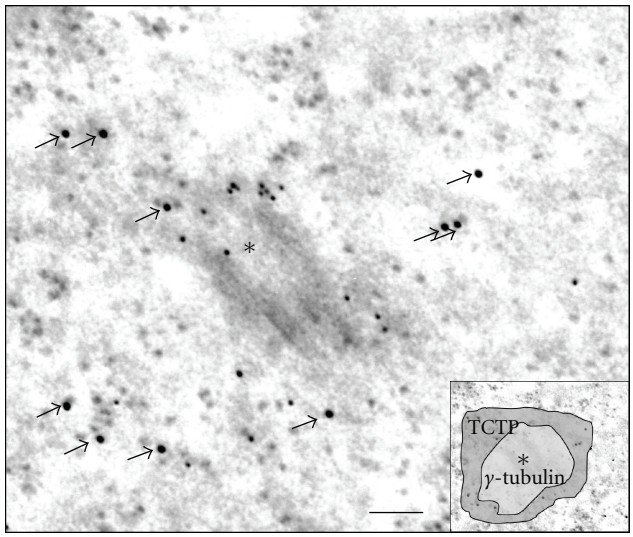


Figure 6: Double labeling of $\gamma$-tubulin and TCTP in the centrosome of *Xenopus laevis* oogonium. Centriole labeled with black asterisk; 18 nm gold particles (black arrows) correspond to the presence of TCTP, small, and 10 nm gold particles around the centriole mark the presence of $\gamma$-tubulin. Inset in the bottom right corner shows the central area around the centriole where $\gamma$-tubulin is present (clear central area), and the TCTP-containing external area of the centrosome (dark grey). Bar is equal to 100 nm.

areas were devoid of $\beta$-tubulin (Figures 4(a) and 4(b); see [5] for details of similar localization of TCTP and MTs in XL2 cells). Electron microscopy immunogold labeling with the anti-XlTCTP antibody showed that TCTP was always localized at a distance of approximately 24 nm (the diameter of a MT) from the MT, but never directly on the MTs (Figure 4(c)). This indicates that TCTP does not associate with MTs directly, but by some intermediates serving as the linkers. Immunolocalization of $\beta$-tubulin and TCTP in mitotic *Xenopus laevis* oogonia showed that in the metaphase, the whole spindle area (detected with anti-$\beta$-tubulin antibody) was heavily stained (Figure 5(a)), while in the telophase, the tubulin-positive midbodies were negative for TCTP (Figure 5(b)) as already shown before in *Xenopus laevis* XL2 cells [5]. To facilitate identification of centrosomes at the electron microscopy level and to identify precisely the areas of the PCM, we detected anti-$\gamma$-tubulin antibody with secondary antibody conjugated with 10 nm gold particles and the anti-XlTCTP antibody with the secondary antibody conjugated with 18 nm gold particles. This double immunostaining showed that $\gamma$-tubulin is present in close proximity of the centriole within an irregular PCM cloud, and that TCTP is present in a layer surrounding the PCM (Figure 6, the inset in the bottom right shows schematically the distribution of $\gamma$-tubulin and TCTP domains around the centriole labeled with asterisk). Thus, the TCTP associates with the PCM of the centrosome, but it does not colocalize with $\gamma$-tubulin.

In conclusion, we show here that TCTP associates with the centrosomes in *Xenopus laevis*, human, monkey, and mouse cells and with the PCM foci in acentriolar mouse oocytes. Moreover, within the centrosomes, the TCTP associates with the external part of the PMC foci but not directly with the centrioles. We also show that TCTP associates with MTs at a distance of about 24 nm. This strongly suggests that the MT-TCTP association requires linkers, whose nature, at present, remains unknown. Though we still do not know the role of TCTP at the centrosomes, considering the fact that the aberrant duplication of centrosomes is a key factor in carcinogenesis (reviewed by [20, 21]), our observations open a new avenue into the study of TCTP/centrosome interactions. Interestingly, p53 was also shown to be associated with the centrosomes [22]. Taking into account the reciprocal negative feedback between TCTP and p53 [4], the potential role of TCTP within the centrosome may involve the antagonistic interaction between these two proteins.

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*Review Article*

# TCTP in Development and Cancer

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The translationally controlled tumor protein (TCTP) is highly conserved among animal species. It is widely expressed in many different tissues. It is involved in regulating many fundamental processes, such as cell proliferation and growth, apoptosis, pluripotency, and the cell cycle. Hence, it is not surprising that it is essential for normal development and, if misregulated, can lead to cancer. Provided herein is an overview of the diverse functions of TCTP, with a focus on development. Furthermore, we discuss possible ways by which TCTP misregulation or mutation could result in cancer.

## 1. Introduction

TCTP was first identified in tumor cells. Since its mRNA has all sequence and structural characteristics of translationally controlled mRNAs, it was named "Translationally Controlled Tumor Protein" [1, 2]. It is also known under many different names, such as histamine releasing factor (HRF), tumor protein translationally controlled (Tpt1), p23, and fortilin. The protein is highly conserved across different species [3], is ubiquitously expressed, but the level of the mRNA varies depending on the cell type [4, 5] and developmental stage [6]. A wide range of extracellular stimuli can rapidly regulate its mRNA level. Examples range from cytokines to calcium levels [7, 8]. Translational regulation of the mRNA adds another layer of TCTP level diversity [9].

TCTP expression seems to be highly regulated at many levels by many distinct mechanisms. It is not surprising that it is associated with an array of different biological activities, such as the cell cycle [3, 10], apoptosis [11–15], cytoskeleton [10, 16, 17], protein synthesis [18], immune response [19], development [6, 20–22], and cancer [11, 23, 24]. In recent years the protein has attracted most attention on account of its role in tumor reversion and its crucial role in development [21, 23]. In this paper we outline what is known so far about TCTP in development with the underlying molecular events and discuss how its misregulation might result in cancer.

## 2. TCTP Promotes Cell Proliferation and Growth

TCTP knockdown studies in *Drosophila* cause lethality in late first-instar larvae and result in reduced cell number, cell size, and organ size [21]. This indicates an effect on cell proliferation and growth, which is regulated mainly by the TOR pathway.

The TOR pathway is regulated by nutrient and energy availability, as well as hypoxia. It integrates signals from many pathways, such as insulin signaling, growth factors, and amino acids. It not only regulates cell growth and proliferation, but also cell motility, cell survival, protein synthesis, and transcription. The pathway is named after the Target of Rapamycin (TOR), a serine-threonine kinase, encoded by the FRAP1 gene. In the presence of growth-promoting signals, receptors on the cell membrane are activated that lead to the activation of the serine-threonine kinase Akt and ultimately TOR. In mammals, the protein TOR is either bound to the protein Raptor (complex TOR1) or to the protein Rictor (complex TOR2). The TOR1 complex is sensitive to the bacterial product rapamycin and is involved in mRNA translation and ribosome biogenesis. The other rapamycin-insensitive complex TOR2 regulates cell survival and the cytoskeleton (reviewed in [25]). It regulates the cytoskeleton by stimulating various proteins,

for example, actin fibers [26]. It also phosphorylates the serine-threonine kinase Akt, which initially leads to TOR activation [27].

TOR1 is activated by an increase in nutrient levels, growth factors, and stress [28]. These extracellular signals activate a cascade of proteins within the cell, leading to the activation of the GTPase Rheb, that ultimately activates TOR1. TOR1 then targets various downstream factors, such as the serine-threonine kinase S6K and the protein 4EBP1 (reviewed in [25]). S6K is known to phosphorylate many proteins. One major target is the S6 ribosomal protein. When nutrients are sparse, the S6 ribosomal protein is bound to the eIF3 complex, which is involved in the initiation of translation by recognizing the 5′ cap structure of mRNAs. mRNAs that contain a 5′ polypyrimidine tract, referred to as 5′ TOP, are important targets of eIF3 translational activation [29, 30]. These transcripts generally encode further ribosomal proteins and translation elongation factors. When the availability of nutrients increases, TOR is activated, causing an increase of S6K. Ultimately, S6 becomes phosphorylated, which causes the eIF3 complex to be released resulting in the activation of translation [31]. Various mRNAs become translated, in particular the mRNAs with a 5′ TOP region that encode proteins involved in translation. This subsequently leads to the production proteins required for translation, overall leading to the amplification of translation.

The other TOR1 target, 4EBP1, is a translation repressor. 4EBP1 binds to the eukaryotic initiation factor 4E (eIF4E), which recruits 40S ribosomal subunits to the 5′ end of mRNAs to initiate translation. Interaction of 4EBP1 and eIF4E results in the inhibition of translation. Upon TOR1 activation, 4EBP1 is phosphorylated, resulting in the dissociation from eIF4E, allowing eIF4E to initiate translation [32].

The entire TOR1 cascade and the increased protein synthesis required the activation of Rheb. Studies in *Drosophila* showed that mutant Rheb resulted in smaller cell sizes and numbers, as observed in the absence of TCTP. It was then determined that TCTP associates with Rhed. This is likely to be conserved between species, as human TCTP was able to rescue *Drosophila* TCTP mutants [21]. This observation directly links TCTP with the TOR pathway, explaining its effect on cell proliferation and growth. In the absence of TCTP, Rhed is no longer active, leading to a decrease in TOR1 activity and ultimately a decrease in protein synthesis in response to external growth-stimulating stimuli. It is known that TCTP responds to many external stimuli. This suggests that the interaction of TCTP with the TOR1 complex might be the reason for the TCTP responsiveness to many external signals [7, 8]. It would be interesting to investigate the connection of TCTP with the TOR2 pathway. Since cells are smaller in TCTP mutants, it is likely that the TOR2 pathway that also regulates the cytoskeleton is involved. To test if TCTP has an effect on the TOR2 pathway, one could analyze the effect of TCTP in the presence of rapamycin. Since rapamycin inhibits the TOR1 activity, it is possible to investigate if the absence of TCTP still has a function on the cell size and growth. If this is the case, it would be interesting to analyze the level of the major TOR2 components in the absence of TCTP. Further studies, for example, with mutants

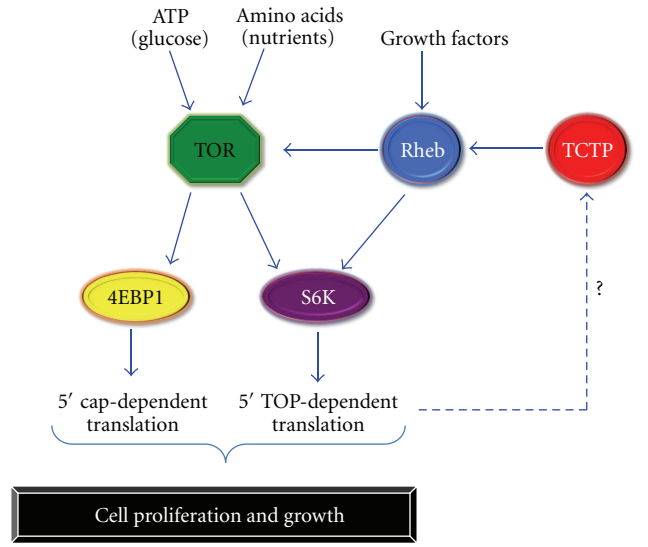


Figure 1: TCTP can activate the TOR pathway and promote cell proliferation and growth.

in a TCTP depleted background could help to elucidate if and where in the TOR2 pathway TCTP could act (Figure 1).

As described above, TOR1 activates the S6K kinase, which activates S6 and leads to translation of mRNAs, in particular of mRNAs that contain the 5′ TOP tract [29, 30]. TCTP mRNA itself contains the 5′-TOP domain [9]. This suggests that TOR1 might activate TCTP translation, via S6K and S6. Since TCTP activates TOR1 by binding to Rheb, the activator of TOR1, it is possible that TCTP not only activates TOR1, but also provides a positive feedback mechanism. Mutating the 5′ TOP domain of TCTP might help to determine if TCTP is actually activated by this route. If this is the case, overexpression of S6K should increase TCTP protein levels, which will in turn promote even more TOR1 activity. S6K could act as a major regulator of TOR1, since it also inactivates the repressor TOR1 by phosphorylation, suggesting a positive feedback mechanism [33]. TCTP could also act in this way, providing a positive feedback mechanism to upregulate TOR. When TCTP is high, it activates TOR1, which in turn leads to the phospporylation of S6 by S6K and increased translation. This might again lead to an increased TCTP protein level that increases TOR1 activation.

Even though abnormal cell proliferation and growth can be explained by TCTP interaction with the TOR pathway, this does not fully explain why development is ultimately arrested. This suggests that TCTP has also a major function that lies outside of the TOR pathway.

## 3. TCTP Inhibits Apoptosis

In the early development of mice, TCTP mRNA and protein levels are significantly increased from embryonic day E3 to E5, when they reach a maximum level. Selective depletion of TCTP in the uterus at E3 resulted in reduced numbers of implanted embryos compared to wild-type embryos [34]. In knockout mice, heterozygous mutants of TCTP

had no obvious developmental effects, but homozygous mutants were lethal between E9.5 and E10.5 [22]. Severe abnormalities became most prominent at E5.5, which is when TCTP level is normally at its highest. Not only did the mice embryos appear smaller, but the epiblast that eventually develops into the fetus also contained a significantly lower cell number. The reason for this was determined to be a misregulation of apoptosis [22].

Apoptosis is a crucial part of the life of a multicellular organism. It is a highly regulated process, resulting in programmed cell death. Insufficient apoptosis may result in accumulation of mutations and uncontrolled cell proliferation, such as in cancer. Apoptosis can be induced by extracellular or intracellular signals and involves the activation of various regulatory proteins that activate the apoptotic pathway. This process is highly regulated, so that apoptosis is not induced unnecessarily, and can even be stopped if the need for apoptosis is no longer required. The intracellular apoptotic pathway is mainly regulated with the help of mitochondria, which supplies the cell with energy. A change in the permeability of the mitochondrial membrane can cause apoptotic proteins to leak into the cell. Pores called mitochondrial outer membrane permeabilization pores (MACs) regulate the permeability of the mitochondrial membrane to apoptotic proteins. Proteins belonging to the Bcl-2 protein family can regulate these MACs [35]. The protein Bax, when activated, dimerizes within the mitochondrial membrane. This dimerization promotes MAC pore formation, causing apoptotic proteins to enter the cell. In contrast, the proteins Bcl-2 and Mcl-1 inhibit MAC formation, preventing the influx of apoptotic proteins into the cell (reviewed in [36]). Apoptotic proteins that can be released via MACs into the cell are generally called small mitochondria-derived activator of caspases (SMACs). These can bind to inhibitors of apoptosis proteins (IAPs) within the cell. IAPs are usually bound to cysteine proteases that are referred to as caspases [37]. These caspases are enzymes that can degrade intracellular proteins, which ultimately cause the degradation of the entire cell. Often, these caspases need to be proteolytically cleaved in order to become active. In addition to SMACs, MAC pores also release the protein cytochrome c. Cytochrome c can then form a complex called apoptosome, by binding to ATP, the apoptotic protease activating factor1 (Apaf1) and procaspase-9. This results in the proteolytic cleavage of pro-caspase 9 into the enzymatically active form caspase 9, overall activating cellular degradation [38].

In a normal cell, the mitochondrial membrane is not permeable to SMACs. As a result, no cytochrome c is in the cell to activate caspase 9. Another class of inhibitor, the IAP proteins, are bound to caspases. Upon an apoptosis-inducing signal, the mitochondrial membrane is permeabilized, releasing SMACs and cytochrome c into the cell. SMACs bind IAPs, which release caspases, and cytochrome c converts caspase 9 to its active form (reviewed in [36]). This results in intracellular digestion and cell death. The necessity for different factors to be exported by the mitochondria shows the high level of regulation. This is not surprising, as a malfunction of the system would be detrimental to the cell.

TCTP also seems to play an important role in controlling the potentially suicidal pathway. It was found to inhibit the proapoptotic protein Bax that promotes MAC pore formation by dimerizing in the mitochondrial membrane. TCTP inserts itself into the mitochondrial membrane, preventing Bax from dimerizing [22]. This prevents MAC pore formation and inhibits any flux of apoptosis promoting factors into the cell [15]. Another study showed that TCTP also binds to Mcl-1. As discussed above, Mcl-1 inhibits MAC formation. Since binding of TCTP was found to stabilize Mcl-1, TCTP increases the block on MAC formation and ultimately prevents apoptosis [13, 14].

It remains to be investigated what happens to TCTP when apoptosis is initiated. It is possible that the TCTP protein is actively degraded or isolated from the system, or that the TCTP mRNA level decreases. In both cases, it is likely that a factor is required for TCTP inactivation. Pull-down studies and promoter analysis when apoptosis is induced could help to find important regulators of TCTP.

## 4. TCTP in Pluripotency and Nuclear Reprogramming

During development cells become committed and differentiate from one cell into many distinct cell types. Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocysts of an early embryo. In contrast to committed or differentiated cells, pluripotent cells can differentiate into any fetal or adult cell type and are capable of self-renewal and unlimited proliferation [39]. These have tremendous potential in medicine, as ES cells could be differentiated into any cell type or even tissue of the body and be used for potential cell replacement therapies.

ES cells are characterized by a particular pattern of gene expression. For example, various genes are upregulated in ES cells and are frequently used as pluripotency markers. Oct4 seems to be an important regulator of pluripotency and differentiation [40]. It represses or activates expression of different genes, which occurs either directly by binding to promoter regions or indirectly by neutralising transcription activators [41]. Oct4, also known as Oct3, is a member of the POU transcription family [42]. These are transcription factors that bind via an octameric sequence to an AGTCAAAT consensus sequence [41]. The gene is expressed in early mammalian embryos, during gametogenesis, in ES cells [43], and occasionally in tumours [44]. After gastrulation, Oct4 becomes silent in mouse and human mammalian somatic cells [45]. In mouse oocytes, Oct4 mRNA is present as a maternal transcript [46] and it is downregulated when development proceeds [47]. It is essential, but not sufficient to maintain cells in an undifferentiated state [48]. During embryonic development, Oct4 is expressed in early blastomeres. Then, it becomes restricted to the inner cell mass, and is down regulated in the trophectoderm and primitive endoderm [47]. Oct4 is widely conserved. Homologues even exist in early amphibian development, where they also act as suppressors of cell fate commitment. Even though so far ES cells have not been derived from amphibians,

the *Xenopus laevis* version of Oct4, Pou91, was able to fully support mouse ES cell self-renewal [49]. This suggests a similar function for Pou91 in pluripotency.

Pluripotency also requires other factors, for example, the leukaemia inhibitory factor (LIF). LIF is a key molecule required for self-renewal and pluripotency in mouse ES cells [50, 51], but not for monkey or human ES cells [52]. It is known to bind to the heterodimer LIF receptor—gp130 and to activate the transcription factor STAT3 by phosphorylation [53]. Interestingly, overexpression of the gene Nanog can bypass the requirement for LIF in mouse ES cells [54]. Nanog is also required for maintaining the undifferentiated state of early postimplantation embryos and ES cells [54, 55], making Nanog an important regulator of pluripotency. There are also other components required, such as bone morphogenic proteins (BMP) that activate the inhibitor of differentiation (Id), which represses differentiation [56]. Another important regulator is Sox2, which cooperatively binds the Oct4 protein and activates genes promoting pluripotency [57], but represses its inhibitors [58].

Despite obtaining the ES cells from blastocysts, ES or ES-cell-like cells can be obtained by nuclear reprogramming, a term introduced to describe the restoration of the embryonic pattern of gene expression [59]. Nuclear reprogramming was first demonstrated in nuclear transfer experiments. *Xenopus laevis* nuclei of differentiated cells were transplanted into enucleated frog eggs. This gave rise to normal fertile adult frogs, illustrating that differentiated cells can become reprogrammed and give rise to an entire new organism [60, 61]. Another way to reprogram nuclei was achieved when cells were fused to each other [62, 63]. Cell fusions with ES cells rejuvenated somatic cells that could differentiate into many different cell types. In these hybrids the silent gene Oct4 was reactivated [64]. Fusion experiments with an increased expression of the pluripotency gene Nanog increased nuclear reprogramming efficiency by 200-fold [65]. Nowadays, the most common way somatic cells are reprogrammed to an embryonic-like pattern of gene expression is by overexpressing different factors, such as Oct4, Sox2, c-Myc, and Klf4 under ES cell culture conditions [66]. Surprisingly, Nanog was not required, even though it seemed to promote nuclear reprogramming in cell fusion experiments [65]. These ES-like cells had normal ES cell morphology, a gene expression pattern typical for normal ES cells and could differentiate into all three germ layers. They were named iPS cells, induced pluripotent stem cells [66]. Even though the generation of iPS cells is a very convenient way to generate ES cells, this approach does not reveal the mechanism underlying nuclear reprogramming. Also, it does not identify novel factors that are involved in this process.

To better understand the process of nuclear reprogramming, nuclear transfer experiments of somatic cells into Xenopus oocytes were carried out. It was found that even human or mouse nuclei could be reprogrammed by frog oocytes and induce an ES cell or ES cell-like pattern of gene expression [67]. For example, genes such as Oct4, Nanog, and Sox2 became transcriptionally active upon nuclear transfer [67]. Using this system, novel molecules were isolated that interact with the promoter region of Oct4. One of these molecules was TCTP. Further functional assays revealed that it in fact TCTP changed the transcriptional level of Oct4 and even Nanog in human nuclei, genes essential for successful nuclear reprogramming [68]. A similar effect of TCTP was found in bovine oocytes, suggesting a conserved function of TCTP in activating pluripotency [69]. TCTP knockout mice have an abnormal number of cells in the epiblast [22]. The epiblast is formed from the inner cell mass of the blastocyst, from which ES cells can be obtained. Since TCTP activates the pluripotency genes Oct4 and Nanog, it is possible that, in the TCTP knockout mice, the epiblast does not develop normally due to misregulation of pluripotency genes such as Oct4 and Nanog.

It would be interesting to determine if TCTP activates also other pluripotency genes such as Sox2 and Klf4. TCTP might promote pluripotency in two different ways, namely, by (1) activating pluripotency genes and (2) inhibiting somatic gene expression. Genomewide studies in the absence of TCTP could help to determine what other genes TCTP regulates. Another important question is whether TCTP is sufficient for nuclear reprogramming and if its overexpression in somatic cells could replace the four reprogramming factors used to make iPS cells. Even if it does not replace these four factors, it could increase the generation of iPS cells, a currently very inefficient process.

Nuclear actin polymerization has been reported to be required for Oct4 activation in *Xenopus laevis* oocytes [70]. Since TCTP has been found to contain an actin-binding site [17], it is possible that it might interfere with pluripotency gene regulation by interfering with actin. Testing actin polymerization in the absence and presence of TCTP, as well as the effect on Oct4, would help to understand any possible interactions required to induce pluripotency. These experiments could also be analyzed Genomewide, which will greatly help to elucidate the underlying network required to establish pluripotency. Using TCTP as bait to pull down interaction partners together with Genomewide Chromatin Immunoprecipitation analysis of TCTP and its interaction partners will also contribute towards understanding how pluripotency is established.

Another protein that has been found to interact with TCTP in Xenopus oocytes is nucleoplasmin Npm1 [71]. Similar to TCTP knockout mice, mice deficient in Npm1 are embryonic lethal and have smaller embryo sizes [72]. Npm1 is a very abundant protein. In fertilized *Xenopus* eggs, it is involved in the decondensation and hence transcriptional activation of the paternal genome provided after normal fertilization by the sperm (reviewed in [73]). It is possible that TCTP not only activates pluripotency genes, but also that it has a role in paternal gene activation by interacting with Npm1. Disturbing the interaction of TCTP and Npm1 could show if TCTP is also involved in this process. But it is possible that pluripotency and paternal and maternal genome activation is actually not that different. After all, when the genome becomes transcriptionally active, it is set as such, so that it can proliferate and differentiate into an entire organism. Hence, zygotic genome activation could be regarded as nuclear reprogramming that occurs naturally

in nature, without the need of nuclear transplantation, cell fusion experiments, or overexpression of a few transcription factors.

## 5. Cell Cycle Regulation of TCTP

The cell cycle describes the stages a cell has to go through to divide and duplicate its genome. In eukaryotes, the cell cycle is divided into four phases: (1) the $G_1$ phase, in which the cell grows and makes sure it is prepared for DNA replication, (2) the S or synthesis phase, where the DNA is duplicated, the (3) $G_2$ phase, in which the cell ensures it is ready for mitosis, and (4) the M phase, in which cell growth stops and the cell divides its DNA and other cellular components giving rise to two cells. There is also an additional phase, which is not part of the cell cycle, $G_0$, in which the cell has exited the cell cycle and has stopped dividing [74]. Since the cell cycle is crucial for the survival of the cell and generation of a multicellular organism, the process is highly controlled. There are many proteins that control each phase and that detect and repair genetic damage, as well as avoiding the propagation of mutations [74]. Any misregulation might result in uncontrolled cell proliferation and ultimately cancer. The key enzymes regulating the progression from one phase into the next are called cyclins and cyclin-dependent kinases. There are also many other proteins, such as the serine-threonine protein kinase polo-like kinase 1 (PLK1) and the protein checkpoint with forkhead and ring finger domains (CHFR).

The protein CHFR is an E3 ubiquitin ligase that can detect microtubule abnormalities. It delays the $G_2$ to mitosis transition when it is exposed to altered microtubules. Microtubules are part of the cytoskeleton and act in mitosis and move the duplicated genomes into the forming daughter cells. CHFR is usually present in an inactive form, unable to carry out ubiquitination. When microtubules are damaged, CHFR becomes activated [75]. CHFR then ubiquitinates PLK1 that results in PLK1 degradation [76]. The kinase PLK1 is required in the late $G_2$ and early mitotic phases. It regulates spindle assembly and centrosome maturation, which is a microtubule-organizing center. PLK1 phosphorylates and activates Cdc25C, which dephosphates and activates the cyclins required for mitosis, the cyclinB/cdc2 complex [77, 78]. Any loss of PLK1 can induce a block in cell cycle progression and lead to apoptosis. PLK1 overexpression is frequently observed in connection with centrosome abnormalities, improper segregation of chromosomes and tumor cells.

Although the TOR pathway might be indirectly involved in cell cycle regulation by responding to growth factors and energy levels and driving cell proliferation, TCTP seems to be involved more directly in the cell cycle. For example, TCTP expression is upregulated upon entry into the cell cycle, but when overexpressed, cell cycle progression is delayed [10]. TCTP also has a tubulin-binding site that allows it to bind to microtubules in a cell-cycle-dependent way. As a result, it is recruited to the mitotic spindle during metaphase, but is released at the $M/G_1$ transition [10]. Furthermore, TCTP interacts with CHFR that interacts with microtubules [79]. Upon depolymerization of the microtubules, CHFR and TCTP interaction is diminished. It has been suggested that this might provide a mechanism by which CHFR senses microtubule abnormalities that results in CHFR activation, PLK1 degradation, and ultimately cell cycle arrest [79]. It would be interesting to determine if CHFR can bind with the same affinity to microtubules in the absence of TCTP, or if it is no longer sensitive to microtubule abnormalities in the absence of TCTP, confirming the proposed model. In addition to binding to CHFR, TCTP can be phosphorylated by the substrate of CHFR, PLK1 [80]. This presumably leads to a decrease in the affinity of TCTP for microtubules or CHFR. When PLK1 phosphorylation sites on TCTP are blocked, a dramatic increase in multinucleated cells is observed suggesting that the completion of mitosis is inhibited [81]. This suggests that TCTP is crucial in cell cycle regulation and that its phosphorylation by PLK1 is required for accurate exit from mitosis. In the TCTP mutants that cannot become phosphorylated, an increase in apoptosis is also observed [81]. Bearing in mind that TCTP is involved in apoptosis, it is possible that PLK1 acts *via* TCTP to inhibit apoptosis. TCTP phosphorylation by PLK1 causes cell cycle progression. It is possible that this modified TCTP might have inhibitory effects on the apoptotic pathway. In this way, TCTP could make sure that when cell cycle progresses no apoptosis is induced. In contrast, if it is not modified by PLK1 during mitosis, it might induce apoptosis via the different routes described above. It would be revealing to investigate the role of the modified TCTP protein in apoptosis.

## 6. TCTP in Cancer

TCTP has been associated with tumorigenesis and cancer since its discovery in tumor cells [1, 2]. It was not until tumor reversion screens that TCTP got attention as a key player in cancer (Figure 2) [11, 23]. Tumor reversion is a process by which some cancer cells lose their malignant phenotype. Studying this process might help to understand how cancer can be inhibited and ultimately lead to a cure. To understand this process on a molecular level, tumor cells were grown in the presence of the H1 parvovirus [23]. This virus preferentially kills tumor cells, which in turn allows for selection of cells that revert back to a normal, nonmalignant phenotype [82, 83]. To identify which genes are most likely to be involved in this process, the level of gene expression was compared between malignant and reverted state. The TCTP gene expression level showed the largest difference between malignant and reverted state. A high level associates with tumorigenesis and a low level with normal cell growth (124 times higher TCTP level in tumor cells versus revertants). This was confirmed in several different tumor cell lines, suggesting that it is a universal gene that is implicated in tumor reversal [23]. Furthermore, knockdown experiments of TCTP in various malignant cell lines increased tumor reversal by approximately 30% [11].

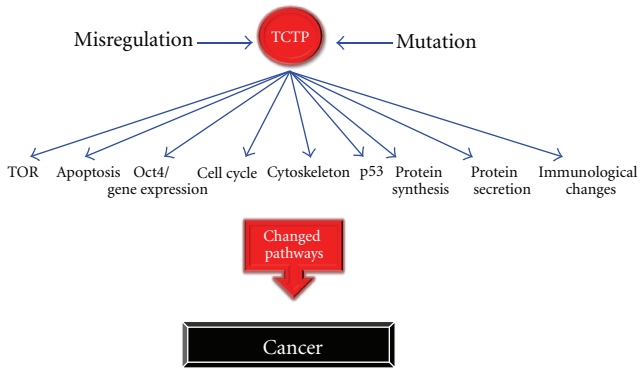


Figure 2: Pathways in which TCTP misregulation or mutations could cause cancer.

The p53 protein is one of the most famous tumor suppressors and is often referred to as the "guardian" of cancer. It is a transcription factor and regulates the transcription of various genes. It can activate the transcription of DNA repair genes when the DNA is damaged, through genes involved in cell cycle and initiate apoptosis by regulating genes such as Bax and Bcl-2 [84]. In response to stress such as DNA damage, it either induces repair genes to repair the damage, cell cycle arrest to prevent the replication of damaged DNA, or induces apoptosis to eliminate potentially malignant cells. Various signals are responsible for whether p53 induces repair, cell cycle arrest, or apoptosis (reviewed in [85]).

To better understand how TCTP levels control cancer, the interaction between TCTP and p53 has been studied in more detail. It was found that TCTP overexpression can lead to p53 degradation. This was accompanied by the observation that p53 was no longer able to induce apoptosis [24]. This suggested that TCTP is an important regulator in the p53 pathway and also links p53 with apoptosis.

MDM2 is a transcriptional target of p53. When overexpressed, MDM2 ubiquitinates and degrades p53, providing a negative feedback mechanism. TCTP was found to inhibit MDM2 autoubiquitination and to promote MDM2-mediated ubiquitination of p53, which ultimately leads to p53 degradation [86]. In addition, p53 was found to downregulate TCTP levels [23] and to promote TCTP exosome secretion [87, 88]. This shows that p53 and TCTP antagonize each other. Similar evidence comes from a different observation. The dsRNA-dependent protein kinase (PKR) increases p53 transcriptional function [89]. Mice depleted of PKR had altered TCTP protein levels. Further analysis showed that PKR directly interacts with TCTP mRNA. This interaction is required for PKR activation [9]. Hence, the presence of PKR might sequester TCTP mRNA and remove free TCTP mRNA from the RNA pool that would otherwise be available for translation. Hence, a higher level of PKR might be associated with lower TCTP protein levels. As PKR activates p53 and counteracts TCTP, it adds another layer of antagonistic control between TCTP and p53. The level of both p53 and TCTP might determine which pathway to choose, cell cycle arrest or apoptosis.

As outlined above, TCTP misregulation has an impact on the TOR pathway, apoptosis, reprogramming and cell cycle. All of these pathways can be implicated in cancer when they do not function correctly. In the TOR pathway, overexpression or mutations enhancing TCTP activity might result in increased TOR activation, leading to enhanced cell growth and ultimately tumor formation. Similarly, alterations in TCTP level might alter the ability of TCTP to inhibit apoptosis. Any TCTP misregulation might prevent damaged cells from being eliminated by apoptosis and in this way promote the survival of cells that might result in cancerous cells. Nuclear transfer experiments have shown that TCTP induces the transcription of pluripotency genes such as Oct4 and Nanog. An increased level of TCTP in normal cells may promote the formation of pluripotent-like gene expression. This might partly reprogram quiescent differentiated cells into pluripotent like proliferating cells. If in addition mutations accumulate in these cells, elevated levels of TCTP might enhance the propagation of these mutated cells. The higher the level of pluripotency transcripts is, the greater is the cell's malignant potential [90]. This suggests that this is also true for a higher TCTP level. Ultimately, this can result in cancer. Misregulation of TCTP might also impact cell cycle progression by interfering with PLK1. PLK1 is overexpressed in a range of human tumors, and PLK1 overexpression is associated with a bad cancer prognosis [91]. Since PLK1 phosphorylates TCTP that is required for cell cycle progression from mitosis, it is possible that an overexpression of PLK1 causes faster TCTP phosphorylation and cell cycle progression. This faster cell cycle progression might result in cell cycle progression even when mitosis is not complete. The resulting daughter cells could in this way inherit not fully replicated genomes. This might result in a vast amount of mutations that might result in cancer. Alternatively, TCTP could be mutated, being irresponsive to PLK1, and have the same effect.

Finally, TCTP is also known to be involved in protein synthesis by acting as a guanine nucleotide dissociation inhibitor for the elongation factor EF1A [18]. Any changes in TCTP level could influence many genes at once and change the status of a cell substantially. Similarly, changes in TCTP will also affect the immune response and ultimately might promote cancer development [19].

## 7. Conclusion

In summary, TCTP is highly conserved and abundant. It is involved in many key biological pathways, such as the TOR pathway, apoptosis, nuclear reprogramming, and cell cycle. It is highly regulated on a transcriptional, translational and protein level. As TCTP is involved in a wide array of biological functions, it is not surprising that any changes to TCTP might result in an array of abnormal phenotypes. Furthermore, abnormal cell proliferation, growth, and survival are probably the most important characteristics of cancer, all of which are regulated by TCTP. Due to this involvement, and its presence in many other pathways, TCTP might be a crucial target for cancer therapies. Some success has already

been reported in this regard [11]. Bearing in mind that TCTP is also a histamine-releasing factor, inhibitors of this pathway were tested for their ability to decrease the number of tumor cells by inhibiting TCTP. In fact, many of such inhibitors were found to kill tumors [11]. However, further studies are required to better understand the function of TCTP in the pathways described before and maybe to reveal further functions. Overall, this will greatly contribute to the understanding of basic molecular pathways and provide further target sites for cancer therapies.

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*Review Article*

# Role of Translationally Controlled Tumor Protein in Cancer Progression

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Translationally controlled tumor protein (TCTP) is a highly conserved and ubiquitously expressed protein in all eukaryotes—highlighting its important functions in the cell. Previous studies revealed that TCTP is implicated in many biological processes, including cell growth, tumor reversion, and induction of pluripotent stem cell. A recent study on the solution structure from fission yeast orthologue classifies TCTP under a family of small chaperone proteins. There is growing evidence in the literature that TCTP is a multifunctional protein and exerts its biological activity at the extracellular and intracellular levels. Although TCTP is not a tumor-specific protein, our research group, among several others, focused on the role(s) of TCTP in cancer progression. In this paper, we will summarize the current scientific knowledge of TCTP in different aspects, and the precise oncogenic mechanisms of TCTP will be discussed in detail.

## 1. Introduction

Translationally controlled tumor protein (TCTP) is a highly conserved multifunctional protein. Since the discovery of TCTP over two decades ago, the expression level of TCTP has been investigated in more than 500 tissues and cell types. Expression levels have been found to vary by nearly two orders of magnitude between different types of tissues [1] with preferential expression in mitotically active tissues [2]. Two mRNA transcripts have been reported to carry the same 5′UTR but different 3′UTR using alternative polyadenylation signals. The solution structure of TCTP from fission yeast also revealed structural similarity with the Mss4/Dss4 protein family. The expression of TCTP is highly regulated both at transcriptional and translational levels in addition to a wide range of extracellular signals. It has been implicated in many cellular processes, such as cell growth, cell cycle progression, apoptosis, malignant transformation, and the regulation of pluripotency. Although TCTP is not a tumor-specific protein, the downregulation of TCTP was found in tumor reversion [3]. Our research group and others also substantiated the link between TCTP deregulation and cancer progression [4–6].

This paper will mainly focus on the biological functions of TCTP and malignant transformation induced by TCTP. Furthermore, the clinical implications of TCTP in human cancers will also be discussed.

## 2. Features of TCTP mRNA and Protein

*2.1. TCTP mRNA.* The human TPT1 gene coding for TCTP spanning about 4.2 kb, consists of six exons and five introns [7]. Two alternative polyadenylation signals at 3′UTR generated two mRNA transcripts that differ in the length of 3′UTR. In all mammalian tissues tested, both types of mRNAs are expressed at different ratios, and the shorter transcript is usually expressed more abundantly [2]. Sequence analyses of TCTP transcripts indicated that the 5′UTR is CG-rich with a high degree of secondary structure. TCTP mRNA has a 5′ terminal oligopyrimidine tract (5′TOP), which is a signature of translationally controlled mRNAs [8]. Although AU-rich

regions and AUUA elements have been identified at the 3′UTR, they do not match classical mRNA instability elements [1].

*2.2. TCTP Protein.* As revealed by sequence alignment of TCTP in more than 30 different species, TCTP is highly conserved over a long-term evolution. A cluster of invariant residues were located on one side of the $\beta$-stranded "core" domain that is important for molecular interactions [1]. Thaw et al. found that the "core" domain displays significant similarity to that of the Mss4/Dss4 protein family upon analyzing the solution structure of TCTP from *Schizosaccharomyces pombe* [1, 9]. TCTP is also a novel small molecular weight (23 kDa) heat shock protein that protects cells from thermal shock by functioning as a molecular chaperone [10].

*2.3. Transcriptional and Translational Regulation.* Previous reports demonstrated that expression of TCTP is regulated at the level of the transcription as well as the translation [2, 11]. TCTP is translationally regulated: abundant TCTP mRNA was found as untranslated mRNP particles [11], an increase in TCTP synthesis under the treatment of transcription inhibitor, actinomycin D [12], a 5′-TOP of TCTP mRNA and its extended secondary structure [8].

Comparing the promoter region of TCTP in human, mouse, rat, rabbit, and dog predicted the transcription factor binding sites of TCTP. Not surprisingly, the promoter regions of TCTP are also highly conserved among these species. Andree et al. further demonstrated that the transcription of TCTP is controlled by cAMP signaling via phosphorylation-dependent activation of CRE/CREB interaction [7]. In our previous study, we utilized chromatin immunoprecipitation-based (ChIP-based) cloning strategy to identify genes potentially regulated by CHD1L (chromodomain helicase/ATPase DNA binding protein 1-like gene) [13]. From this strategy, we isolated 35 CHD1L-binidng loci and characterized a specific CHD1L-binding motif (C/A-C-A/T-T-T-T). Two CHD1L-binding motifs have been identified at −748 bp and −851 bp in the 5′-flanking region of TCTP [13]. Importantly, we have demonstrated that the binding of CHD1L to the promoter region of TCTP dramatically activates the transcription of TCTP.

## 3. Biological Functions

*3.1. Growth and Development.* A knockout mouse approach had been used to investigate the role of TCTP in development. As demonstrated by Chen et al., heterozygous mice ($TCTP^{+/-}$) were viable, fertile, and morphologically similar to their wild-type littermates, while the homozygous ($TCTP^{-/-}$) were embryonic lethal. Moreover, $TCTP^{-/-}$ embryo at embryonic stage day 5.5 (E5.5) suffered from reduced cell numbers and increased apoptosis and subsequently died around E9.5–10.5 [14]. This suggests that TCTP is essential for normal development. The human TCTP (hTCTP) protein sequence is 50% identical with *Drosophila* TCTP (dTCTP) [15]. They also indicated that silencing of dTCTP by RNA interference resulted in the reduced cell size, cell number, and organ size. Also, as Rheb is an important regulator in TSC-mTOR pathway, TCTP might function as a growth-regulating protein by the stimulation of GDP/GTP exchange of human Rheb (hRheb) via binding to hRheb [15]. On the contrary, Rehmann et al. argues that TCTP does not act as a guanine nucleotide exchange factor (GEF) of Rheb [16]. Therefore, additional experiments are essential to further substantiate the role of TCTP in cell growth regulation through the TSC-mTOR pathway.

*3.2. Regulation of Cell Cycle and Apoptosis.* Gachet et al. demonstrated that TCTP interacts with microtubules during G1, S, G2, and early M phase of the cell cycle [17]. During mitosis, it binds to the mitotic spindle and detaches from the spindle during the metaphase-anaphase transition. When TCTP is overexpressed in bovine mammary epithelial cells, rearrangement of microtubule and growth inhibition can be observed [17]. Two-hybrid screening methods identified TCTP as a substrate of polo-like kinase (Plk), which is involved in the formation and function of bipolar spindles and Plk phosphorylates TCTP on two serine residues [18]. Abolishing Plk phosphorylation on TCTP-induced mitotic defects and high incidences of apoptosis, indicating that phosphorylation of TCTP on two serine residues by Plk plays an important role in cell mitosis [18].

*3.3. Regulation of Self-Renewal and Pluripotency.* Homeodomain transcription factors Oct4 and Nanog have been identified as master regulators of stem cell self-renewal and pluripotency [19]. Oct4 appears to regulate cell fates in a quantitative fashion and maintain a critical concentration to sustain embryonic stem (ES) cell self-renewal [20]. Proteins associated with the regulatory region of the mouse *oct4* gene can be isolated and identified by mass spectrometry [21]. Using this strategy, TCTP was found to bind to the promoter region of *oct4*. Although *tpt1* transcript depletion can inactivate the transcription of both *oct4* and *nanog*, TCTP binds only to the *oct4* promoter as determined by ChIP analysis in mouse ES cells [21]. It has also been suggested that TCTP could regulate *oct4* by directly binding to its proximal promoter. However, TCTP might also regulate *nanog* indirectly or binds to its distal promoter. These data suggest that TCTP is a potential regulator of self-renewal and pluripotency.

## 4. Malignant Transformation by TCTP during Cancer Progression

Although there are many distinct types of human cancers, six essential alterations to normal cells are believed to define the progression of most human malignancies: they are evasion of apoptosis, sustained angiogenesis, accelerated cell cycle progression, tissue invasion and metastasis, self-sufficiency in growth signals, and insensitivity to antigrowth signals.

Therefore, the information described below provides us with some insights into the oncogenic role of TCTP.

*4.1. Differential Expression of TCTP in Cancer.* Independent studies indicated that TCTP is preferentially expressed in cancer. In human colon cancer, the level of TCTP mRNA was detected in three human colon carcinoma cell lines (SNU-C2A, SNU-C4, and SNU-C5). The expression levels were not equal among these cell lines. SNU-C5 with the highest expression grew at the fastest rate; however, SNU-C2A with the lowest expression grew at the slowest rate [4]. Higher expression level of TCTP was also observed in prostate cancer specimens compared to normal prostate tissues [5]. In hepatocellular carcinoma (HCC), the expression level of TCTP was detected in a retrospective cohort of 118 HCC patients. As a result, TCTP was found to be significantly upregulated in tumor tissues when compared to their adjacent noncancerous tissues. Overexpression of TCTP (defined as 2 fold increase) was detected in 40.7% of HCC specimens [6].

*4.2. Antiapoptosis.* Overexpression of TCTP was detected in many types of tumors and its downregulation decreases the viability of those cells [3]. These suggest that TCTP is a prosurvival factor in normal and cancer cells.

Myeloid cell leukemia 1 (Mcl-1) is an antiapoptotic protein identified as an early gene induced during differentiation of ML-1 myeloid leukemia cells. It is a member of Bcl-2 family that plays a pivotal role in animal development. Zhang et al. found that TCTP interacts with Mcl-1, but not any other Bcl-2 family member. Further, the depletion of Mcl-1 rapidly destabilized TCTP in an osteosarcoma cell line U2OS, supporting the conclusion that Mcl-1 serves as a chaperone of TCTP, binding and stabilizing TCTP *in vivo* [22]. On the contrary, Liu et al. suggest that TCTP may also serve as a molecular chaperone and cofactor of Mcl-1, in which the association between TCTP and Mcl-1 is essential for both to function [23].

It has been reported that TCTP interacts with Bcl-xL, an antiapoptotic protein that maintains the integrity of the mitochondrial membrane [24]. They found that the N-terminal region of TCTP is responsible for its interaction with the Bcl-xL BH3 domain, which is critical for eliciting antiapoptotic properties. They also proposed that TCTP might inhibit T-cell apoptosis by preventing the phosphorylation/inactivation of Bcl-xL. More recently, the crystal structure of TCTP provides new insights into its antiapoptotic activity. The H2-H3 helices of TCTP share a structural similarity to the H5-H6 helices of Bax [25]. Mutation of residues (E109 and K102) close to the turn between the two helices H2 and H3 of TCTP reduces the antiapoptotic effect of TCTP on Bax-induced apoptosis, indicating that H2-H3 helices of TCTP play an important role in the inhibition of apoptosis. Despite the lack of evidence to support the binding between TCTP and Bax [22, 23, 25], Susini et al. suggested that the anchorage of TCTP into the mitochondrial membrane could inhibit the dimerization of Bax and subsequent Bax-induced apoptosis.

*4.3. Mitotic Defects and Chromosome Missegregation.* In mitosis, APC is activated by binding to Cdc20, and this is dependent on high Cdk1 activity [26]. Subsequently, the active APC recognizes securin and cyclin B, thereby provoking their degradation. Degradation of cyclin B inactivates Cdk1, which subsequently permits mitotic exit [27]. The microtubule binding activity and Plk phosphorylation sites indicate that TCTP is an important gene in the regulation of mitotic progression [17, 18]. As reported in our previous study, the role of TCTP in cell cycle progression has been fully investigated by overexpressing TCTP in HCC cell lines. As a result, TCTP has no obvious effect on G1/S transition; however, when cells were released after synchronization at the prometaphase, an accelerated mitotic exit was observed in TCTP-overexpressing cells [6]. Mechanistic study demonstrated that TCTP promoted the ubiquitin-proteasome degradation of Cdc25C during mitotic progression, which caused the failure in the dephosphorylation of Cdk1-Tyr15 and decreased Cdk1 activity. As a consequence, the sudden drop of Cdk1 activity in mitosis induced a faster mitotic exit and chromosome missegregation, which led to chromosomal instability (Figure 1). We did not observe any obvious difference in cyclin B1 expression level between control and TCTP overexpressing cells, suggesting that the TCTP-mediated faster mitotic exit might not be related to APC-mediated degradation of cyclin B1. Xenograft experiments further supported our notion that the overexpression of TCTP could induce mitotic defects and chromosome missegregation [6].

*4.4. Migration and Metastasis.* Metastasis is the final step in solid tumor progression and is the most common cause of death in cancer patients [28]. Metastasis is a multistep process, all of which must be successfully completed before giving rise to a metastatic tumor. It has been reported that TCTP is preferentially expressed in colon cancer cell lines (LoVo, SW620) with highly metastatic potentials. Depletion of TCTP by shncRNA-TCTP in LoVo cells significantly reduced the number of the hepatic surface metastases in nude mice [29]. Recently, our group has studied the motile and invasive capabilities of TCTP *in vitro* and *in vivo*. As a result, the number of invaded cells was significantly increased in TCTP-overexpressing cells. An experimental metastasis assay was used to examine the metastatic nodules formed in the livers of SCID mice after inoculation with TCTP-overexpressing cells. Cells were injected through the tail-vein of SCID mice, metastatic nodules were counted at 8 weeks after injection. The number of metastatic nodules on the surface of the liver was significantly higher in mice injected with TCTP-transfected cells.

## 5. Clinical Implication of TCTP in Human Cancers

Overexpression of TCTP was found in different types of cancers, including colon cancer [4], prostate cancer [5], and liver cancer [6]. In our previous study, overexpression of TCPT was detected in 40.7% (48 of 118) of HCC cases. Clinically, overexpression of TCTP was significantly

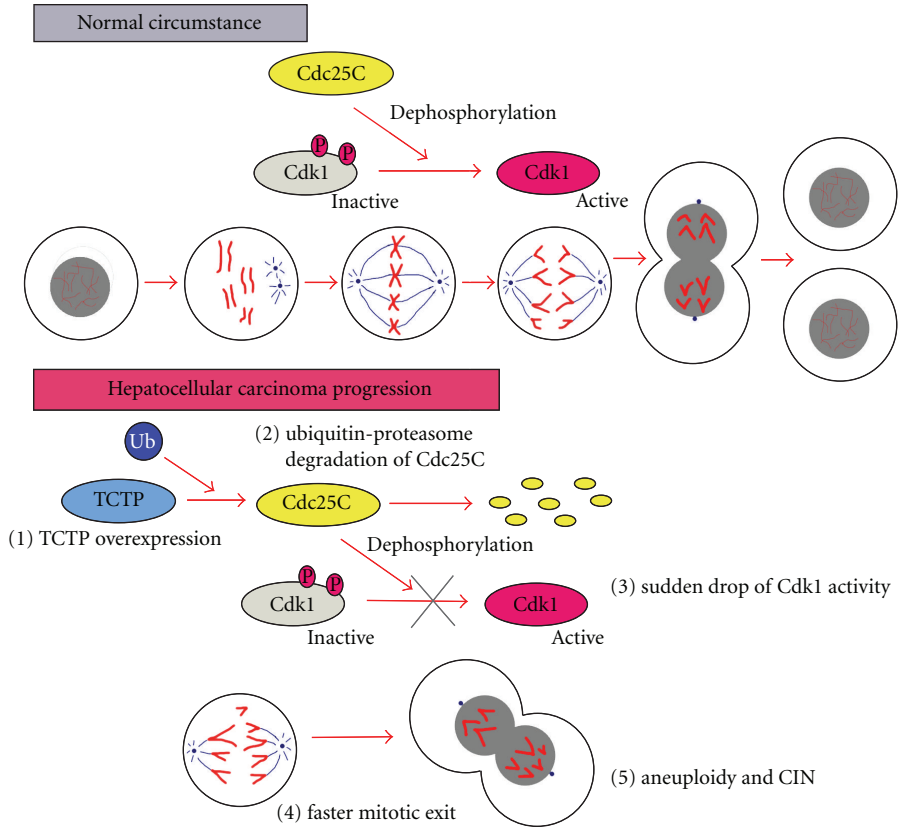


Figure 1: Mechanistic diagram showing the effect of abnormal regulation of TCTP/Cdc25C/Cdk1 pathway in HCC development. (Upper panel) Under the normal mitotic progression, Cdc25C activates Cdk1 by the dephosphorylation of Thr14 and Tyr15 in Cdk1. The level of active Cdk1 is a key factor for maintaining the mitotic state and functions as a key switch for cell division. (Lower panel) During the HCC development, TCTP is overexpressed in over 40% of HCC cases. Overexpression of TCTP promotes the ubiquitin-proteasome degradation of Cdc25C, which leads to the failure in the dephosphorylation of Cdk1 on Tyr15 and decreases Cdk1 activity. As a consequence, the sudden drop of Cdk1 activity in mitosis induces a faster mitosis exit and chromosome missegregation, which leads to aneuploidy and CIN, finally causing cancer development.

associated with the advanced tumor stage and overall survival time of HCC patients. TCTP was also determined as an independent marker associated with poor prognostic outcomes [6]. Moreover, our recent study also indicated that the overexpression of TCTP was significantly associated withextrahepatic metastases (e.g., bone, lymph node, and kidney) among HCC patients.

By comparing the proteome of a melanoma cell line (MeWo) and their chemoresistant counterpart, TCTP was also found to be one of the proteins preferentially expressed in chemoresistant melanoma cell lines [30].

## 6. Conclusions and Future Directions

Due to the ubiquitous expression and high-degree conservation, TCTP protein underlines its important functions in the cell. An increasing number of research investigations are being conducted in this area, particularly into the effect of TCTP during cancer progression. It is implicated in cell growth, cell cycle progression, apoptosis, and regulation in pluripotency. Although TCTP is not tumor-specific, preferential expression of TCTP in different types of cancer underlines the importance of TCTP in cancer progression. As summarized in this paper, TCTP mainly exerts its tumorigenic function via inhibiting apoptosis, accelerating mitotic exit, inducing invasion and metastasis, and so on. By using molecular biological techniques, we demonstrated a molecular pathway, TCTP/Cdc25c/Cdk1, which plays an important role in hepatocarcinogenesis by accelerating mitotic progression and inducing CIN (Figure 1). CIN is a hallmark of many types of human cancers and is significantly associated with poor prognosis. Thus, characterization of this novel pathway will greatly facilitate our insights into the link between aneuploidy cancer development. To better understand the oncogenic mechanism of TCTP, our current work is focusing on the DNA-binding activity of TCTP and identifying its specific binding motifs. Future research on the regulatory network of TCTP will improve our understanding of this oncogene and may ultimately contribute to the development of more accurate treatment modalities.

## Conflict of Interests

The authors declare that they have no conflict of interests.

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*Research Article*

# Sumoylation of Human Translationally Controlled Tumor Protein Is Important for Its Nuclear Transport

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Translationally controlled tumor protein (TCTP) lacks nuclear bipartite localization signal sequence; yet TCTP is present abundantly in the nucleus. At present it is not known how TCTP gets transported to the nucleus. Sequence analyses showed that all TCTPs described to date have putative small ubiquitin-like modifier (SUMO) motifs. Since SUMO modification plays an important role in the nuclear transport of proteins, we evaluated whether SUMO motifs are important for transport of TCTP into the nucleus. We show that TCTP exists in sumoylated form in cytoplasm and nucleus of mammalian cells. Point mutation of lysine residue in the SUMO motif compromised the ability of TCTP to get sumoylated *in vitro*. When cells were transfected with FLAG-tagged mutated TCTP, nuclear transport of TCTP was inhibited confirming that sumoylation is critical for the nuclear transport of TCTP. Our previous studies demonstrated that TCTP can function as an antioxidant protein in the nucleus. When we mutated TCTP at the SUMO motif the antioxidant function of TCTP was compromised. Results presented in this study thus show that sumoylation plays an important role in the transport of TCTP into the nucleus where they function as antioxidant protein.

## 1. Introduction

TCTP is a growth-associated protein ubiquitously present in wide variety of organisms from yeast to mammals [1–3]. In fact it is one of the 20 most abundantly expressed proteins in the cell. TCTP was initially identified in an Ehrlich ascites tumor cell line, hence the name [4]. Subsequently, TCTP was demonstrated to be present in almost all normal cells [5]. Despite the ubiquitous nature of TCTP, its exact cellular function is not clear and the true function of TCTP is still being debated. Interestingly, however, dysregulation of TCTP has been shown to be associated with several disease conditions such as cancer [6], Alzheimer's disease [7], and allergy [2, 8] suggesting an important role for TCTP in the physiological homeostasis of cells. Some of the other important cellular functions attributed to TCTP include calcium binding [9, 10] tubulin binding [11, 12], and antiapoptotic function [13, 14].

Several lines of studies suggest that expression of TCTP is upregulated by a variety of stress conditions such as oxidative stress, heat shock, and exposure to heavy metals. For example, TCTP is upregulated over 12-fold in hypoxic HeLa cells [15] and HepG-2 cells [16]. Similarly, oxidative stress induced by DTT and hydrogen peroxide ($H_2O_2$) can induce highly elevated levels of TCTP expression in yeast cells [1] and in human lung epithelial cells [17]. Our recent studies showed that TCTP can function as a potent antioxidant protein [18] in addition to chaperone-like activity [19] in the cell.

TCTP is a heat stable protein present mainly in the cytoplasm [5]. However, recent findings show that oxidative stress [20] and glucose stimulus [21] translocate TCTP to the nucleus. In addition, treatments with certain allochimeric molecules also result in the enrichment of TCTP in the nucleus [22]. Therefore, it is intriguing how TCTP gets transported into the nucleus. One of the ways by which proteins enter the nucleus involves binding to small molecular weight transporter proteins, such as SUMO, which covalently modifies (sumoylation) the protein [23]. Such covalent modification of cellular proteins by SUMO plays a central role in regulating a variety of cellular processes including nuclear transport, formation of subnuclear structures, regulation

of transcriptional activities, DNA binding abilities of transcription factors, signal transduction, stress responses, cell-cycle progression, protein-protein interactions, and protein stability [24]. At present we do not know the clear function of TCTP in the nucleus. Given that TCTP might function as an antioxidant protein [18, 19], in the present study we asked the question whether sumoylation of TCTP has any significant role in the nuclear transport and protecting cell from damage during oxidative stress.

## 2. Materials and Methods

*2.1. Sequence Analysis.* TCTP sequence cloned from human bone eosinophilic granuloma cell line was analyzed to determine whether it contains any Small Ubiquitin-like Modifier (SUMO) motifs using SUMOplot software available in ExPASy proteomic tools.

*2.2. Evaluating the Expression of TCTP in the Cytoplasm and Nucleus of Mammalian Cells.* Human bone eosinophilic granuloma cell line (CRL-7802) obtained from ATCC (Manassas, VA) was cultured in DMEM supplemented with 10% FBS medium until it reached 100% confluence. Cytoplasmic and nuclear fractions were prepared using a kit purchased from Active Motif (Carlsbad, CA). Briefly, cells were washed once with 5 mL of ice-cold phosphate-buffered saline containing phosphatase inhibitors before removing them from the plate with Trypsin-EDTA. Approximately $1 \times 10^6$ cells were resuspended in 500 $\mu$L 1x hypotonic buffer and incubated on ice for 15 min. 25 $\mu$L detergent was then added and vortexed for 10 seconds followed by centrifugation at 12000 rpm for 30 seconds at 4°C. The supernatant (cytoplasmic fraction) was stored at −80°C until use. The pellet was resuspended in 50 $\mu$L of complete lysis buffer and vortexed for 10 seconds followed by incubating on ice for 30 min. The suspension was then centrifuged at 12000 rpm for 10 minutes at 4°C. The supernatant containing nuclear fraction was stored at −80°C until use. The protein concentrations of cytoplasmic and nuclear fractions were determined using a BCA kit (Thermo Fisher Scientific, Rockford, IL). Levels of TCTP expression in cytoplasmic and nuclear fractions were determined by western blot using polyclonal anti-TCTP purchased from MBL laboratories (Nagoya, Japan).

*2.3. Detecting the Presence of Sumoylated TCTP in the Cellular Fractions.* TCTP was immunoprecipitated using Seize X protein A immunoprecipitation kit obtained from Thermo Scientific Pierce Biotechnology (Cat.No. 45215). Briefly, 1 mg/mL of rabbit anti-TCTP antibody or preimmune rabbit serum was first allowed to bind to immobilized protein A columns for 15 minutes and washed with binding/wash buffer for 5 times in a microcentrifuge at 3500 rpm for 1 minute. In some studies, anti-FLAG monoclonal antibodies (Sigma, St. Louis, MO) were used to immunoprecipitate FLAG-labeled TCTP from cell preparations. Antibodies were first crosslinked to protein A using a DSS cross-linker before using in immunoprecipitation. For immunoprecipitation, 50 $\mu$g of cytoplasmic and nuclear fractions were incubated with the crosslinked antibodies for 1 hour at room temperature. The unbound proteins were removed by washing five times with wash buffer (Thermo Fisher Scientific). The bound proteins were eluted with 200 $\mu$L of immunopure elution buffer (Thermo Fisher Scientific). Eluted samples were resolved on 12% SDS-PAGE and transferred to nitrocellulose membrane and probed with 1 : 1000 rabbit polyclonal anti-SUMO antibodies (Imgenex, San Diego, CA) for 1 hour at room temperature. After washing, the membrane was incubated with 1 : 5000 goat anti-rabbit IgG coupled to HRP for 30 minutes at room temperature and signal developed using an ECL kit (Amersham, Pharmacia Biotech. Piscataway, NJ).

*2.4. TCTP-Ubc9 GST Pull-Down Assay.* Ubc9 gene cloned in pGEX-5X-1 vector at SmaI and BamHI sites was obtained from Dr. V. G. Wilson (Department of Medical Microbiology and Immunology, Texas A&M University System Health Science Center, College Station, TX). Both GST-Ubc9 fusion protein and GST alone were expressed in BL21 (DE3) and purified by glutathione-sepharose columns (Clontech, Palo Alto, CA). His-tagged-TCTP was expressed in BL21 (DE3) carrying pLysS plasmid and purified by cobalt metal affinity chromatography (Clontech). For the binding assay, approximately 2 $\mu$g of GST alone or GST-Ubc9 fusion proteins was prebound to glutathione-sepharose beads by incubation for 1 hour at room temperature in 0.5 mL binding buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 2% bovine serum albumin). Two $\mu$g of purified TCTP was then added, and incubation was continued for 1 hour. The beads were washed five times with wash buffer (10 mM Tris-HCl, pH 8, 140 mM NaCl and 0.025% $NaN_3$). Bound proteins were recovered by eluting with glutathione and were analyzed by western blotting using monoclonal anti-His tag antibody (Qiagen, Valencia, CA). Signals were detected using an ECL substrate kit (Amersham) and signal intensity quantitated using NIH image software.

*2.5. In Vitro SUMO-1 Conjugation Assay.* In order to test SUMO-1 modification of TCTP, HeLa cell extract containing SUMO-1 activating enzymes, UBA2/AOS1 was prepared as described previously [25]. Briefly, five $\mu$g of purified His-tagged TCTP protein was incubated with 5 $\mu$g of HeLa cell extracts in a 100 $\mu$L reaction, containing an ATP-regenerating buffer (50 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 2 mm ATP, 10 mM creatine phosphate, 3 units/mL creatine kinase, and 0.5 unit/mL inorganic pyrophosphatase), 6 $\mu$g of purified SUMO-1, and 1 $\mu$g of ubc9. Reactions were incubated at 37°C for 2 hours. Control reactions had one or more of the components omitted and replaced by additional buffer. The reaction was terminated by the addition of SDS-sample buffer and the reaction products were analyzed by western blotting with monoclonal anti-His tag antibody (Qiagen) by ECL method.

*2.6. Construction of Wild-Type TCTP and Mutant TCTP Expression Vectors.* The open reading frame (ORF) of wild-type TCTP from human bone eosinophilic granuloma was

cloned into a pFLAG vector (Sigma). The forward PCR primer corresponded to the beginning of ORF of TCTP with the addition of an upstream in-frame *HindIII* restriction site (5′ CCCAAGCTTATGATTATCTACCGGGACCTC3′). The reverse primer corresponded to the 3′ end of TCTP ORF flanked by *BamHI* restriction site (5′ CGCGGATCCTTA-ACATTTTTCCATTTTTAA3′). PCR parameters were 95°C of denaturation for 30 seconds, 55°C of primer annealing for 30 seconds, 72°C of primer extension for 30 seconds, and the cycle was repeated for 30 times. A final extension of 5 minutes was performed at 72°C before storing the samples at 4°C. PCR products obtained were digested with HindIII and BamHI enzymes and ligated to similarly digested pFLAG mammalian expression vector. Mutant TCTP (Lys at aa164 mutated to Arg) was prepared using site-directed mutagenesis kit purchased from Stratagene (La Jolla, CA). Primer 1 corresponded to nucleotide 487-507 (TTTAGGGATGGTT-TAAAAATG) of TCTP ORF to revert A residue at 491 to the G residue and a Sca I restriction site (5′AGTACT3′) was added upstream to primer 1. Primer 2 corresponded to nucleotide 466-486 (5′GAAAATCATATATGGGGTCAC′) of TCTP ORF. PCR parameters were 94°C for 4 min, 50°C for 2 minutes, and 72°C for 2 minutes. Followed by 8 cycles of 94°C for 1 minute, 56°C for 2 minutes, and 72°C for 1 minute. A final extension of 5 minutes was performed at 72°C. Following PCR, standard digestion, polishing and ligation was performed as recommended by manufacturer's protocol. Sequencing of both the DNA strands was done to confirm the authenticity of the wild-type and mutant TCTP sequences cloned in pFLAG vector.

*2.7. Expression of Wild-Type and Mutant TCTP Constructs in Cos1 Cell Line.* Cos1 cells purchased from ATCC were cultured in either 6- or 96-well tissue culture plates until they reached ~90% confluence. Cells were then transfected with pFLAG-TCTP or pFLAG-mutant TCTP using Lipofectamine 2000 or Oligofectamine transfection reagent (Invitrogen, Carlsbad, CA) as per the manufacturer's instructions. After 48 hours following transfection, cells were collected to prepare the cytoplasmic and nuclear fractions as described above. Expression of Flag-tagged constructs in these preparations was analyzed by western blotting with 1 : 1000 mouse anti-Flag monoclonal antibodies conjugated to HRP (Sigma). The blots were developed using an ECL method (Amersham).

*2.8. Small Interfering RNA In Vivo Gene Silencing Assay.* The siRNAs of TCTP and laminin A/C were synthesized at Dharmacon Research Inc. (Lafayette, CO). The target sequence of TCTP used for designing siRNA was from nucleotide 121-141 (5′AAGGTAACATTGATGACTCGC3′; sense siRNA, 5′-GGUAA CAUUGAUGACUCGCdTdT-3′; antisense siRNA, 5′-GCGAGUCAUCAAUGUUAC CdT dT-3′). For lamin A/C the target sequence (cDNA) was 5′-CTGGACTTCCAGAAGAACA-3′; sense siRNA, 5′CU GGACUUCCAGAAGAACAd Td T3′; antisense siRNA, 3′GACCUG AAGGUCUUCUUGU-5′. All procedures were performed under RNAse-free environment, using RNAse-free water. Approximately, $10^4$ cells/mL of human eosinophilic granuloma cells placed in 24-well plates were transfected with a final concentration of 50 nM of siRNA duplexes using Oligofectamine reagent as described above. Seventy-two hours after the transfection, cells were collected, lysed by addition of 100 $\mu$L of SDS gel loading buffer, and subjected to SDS-PAGE and western blot analysis, using anti-Lamin A/C (Santa Cruz Biotechnology, Inc.) and anti-TCTP (MBL) antibodies.

*2.9. $H_2O_2$ Tolerance Bioassay.* Effect of oxidative stress in Cos1 cells was measured as described previously by Kuner et al. [26]. Briefly, Cos1 cells transfected with pFLAG-TCTP, pFLAG-mutant TCTP or human granuloma cells transfected with RNAiTCTP, or RNAiLamin were incubated with 100 $\mu$M $H_2O_2$ for 24 hours in culture. Following incubation, the number of live cells was determined using a cell counting kit purchased from Dojindo Molecular Technologies (Gaithersburg, MA). Briefly, 10 $\mu$L of the tetrazolium salt dye was added to the cells cultured in 96-well tissue culture plates and incubated for 4 hours. After incubation, the absorbance was measured at 450 nm using a microplate reader.

*2.10. Statistical Analysis.* Statistical analysis was performed using a Mann-Whitney *U* rank sum tests using SigmaStat 2.0 (Jandel Scientific Software, San Rafael, CA).

## 3. Results

*3.1. Sequence Analysis of TCTP.* Sequence analysis showed that TCTP cloned from human bone eosinophilic granuloma cells has a putative SUMO motif at aa 163-166. A comparison of the TCTP sequences from other species of organisms also showed the presence of putative SUMO motif. Predicted SUMO motifs in the TCTP sequence of other organisms are listed below: mouse TCTP (aa 163-166), rabbit TCTP (aa 163-166), yeast TCTP (aa 159-162), *Schistosoma mansoni* TCTP (aa 161-164), *S. japonicum* TCTP (aa 161-164), *S. haematobium* TCTP (aa 121-124), *Brugia malayi* TCTP (aa 107-110), *Wuchereria bancrofti* TCTP (aa 107-110), and *Onchocerca volvulus* TCTP (aa 107-110).

*3.2. TCTP Binds to Ubc9 In Vitro.* In order for TCTP to be sumoylated, an enzyme called Ubc-9 will have to bind to TCTP first which then catalyzes the binding of TCTP to SUMO-1 [25–28]. To determine whether the sumoylation motif in TCTP is active, we first performed GST Pull-Down assays using bacterially expressed GST-Ubc9. His-tagged TCTP purified by metal affinity column was mixed with GST-Ubc9 and affinity purified in GST column. Ubc9-bound protein was then probed with anti-His antibodies. Our results show that approximately 20% of the TCTP that passed through the column bound to GST-Ubc9 suggesting that TCTP can bind to Ubc9, an enzyme critical for sumoylation (Figure 1(a)). A similar Pull-Down assay with GST alone did not bind TCTP confirming that the binding of TCTP to Ubc-9 is specific.

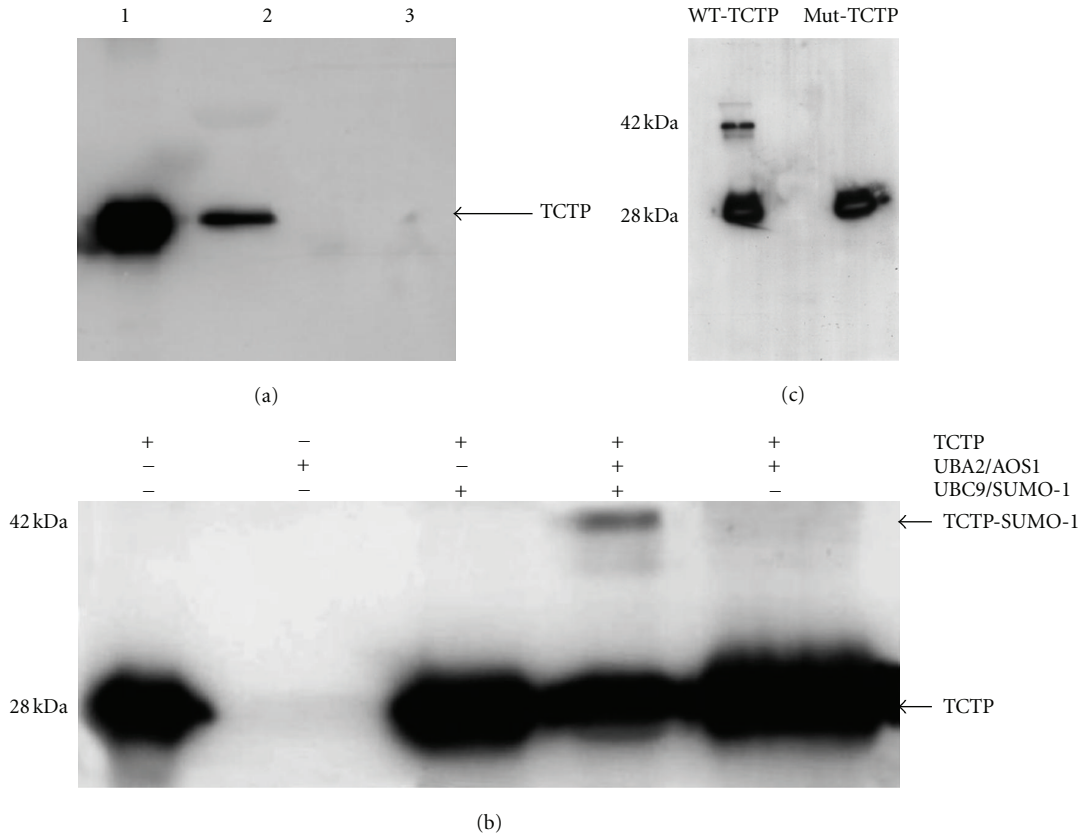


Figure 1: TCTP can be sumoylated *in vitro*. GST Pull-Down assay (a). Proteins were resolved on a 12% SDS-PAGE and transferred to a nitrocellulose membrane and probed with anti-HIS antibody. Lane 1: 5 $\mu$g of rTCTP, Lane 2, shows the amount of TCTP that bound to the GST-Ubc9 enzyme, and Lane 3 shows the amount of TCTP that bound to GST alone control. These results show that TCTP strongly binds to Ubc9 enzyme. *In vitro* sumoylation assay (b). Proteins were resolved on 12% SDS-PAGE and transferred to nitrocellulose membrane and probed with anti-His or anti-TCTP antibody to detect TCTP. Note the high molecular weight product (TCTP-SUMO-1) in Lane 4, where the incubation mixture contained TCTP, UBA2/AOS1, and Ubc9/SUMO. Deletion of any one of these products from the reaction mixture resulted in the absence of this high molecular weight band (Lanes 1, 2, 3, and 5). Mutation studies (c), The lysine residue (aa 164) in the TCTP was mutated to arginine. When such mutated TCTP was subjected to *in vitro* sumoylation, SUMO-1 failed to bind to mutated TCTP, whereas wild-type TCTP could be sumoylated under the same conditions.

*3.3. SUMO-1 Is Covalently Conjugated to TCTP In Vitro.* Ubc9 was recently shown to act as an E2-conjugating enzyme that conjugates SUMO-1 to target proteins instead of ubiquitin [27, 28]. Prosite scan analysis showed that TCTP proteins have either one or two potential sites, where SUMO-1 can be conjugated. To test whether TCTP is a substrate for SUMO-1 modification by Ubc9, we used an *in vitro* reaction system described previously [24]. Following the reaction, a western blotting analysis was performed using anti-TCTP antibodies (Figure 1(b)) or anti-His antibodies (data not shown) to identify TCTP in the reaction mixture. Binding of SUMO-1 to TCTP increases the molecular mass and hence the sumoylated TCTP (~42 kDa) will migrate slower compared to native TCTP (~28 kDa) (Lane 4, Figure 1(b)). The higher molecular weight TCTP appeared only in the presence of SUMO-1, Ubc9, and the SUMO activating enzymes. Leaving out of any component in the reaction mixture failed to produce the high molecular weight TCTP (Figure 1(b)) suggesting that TCTP is a substrate for SUMO-1 conjugation that is mediated by Ubc9. Mutated TCTP (K164R) could not be sumoylated *in vitro*; however, the wild-type TCTP under the same condition was easily sumoylated (Figure 1(c)). These findings thus suggested that the lysine residue (Lys 164) in the SUMO motif of TCTP is critical for its sumoylation. Overall these findings confirmed that TCTP can be sumoylated.

*3.4. TCTP Is Present in the Nucleus and Is Sumoylated.* Analysis of the cytoplasm and nuclear fractions of the human eosinophilic granuloma cell line shows that TCTP is present in both cytoplasm and nucleus (Figure 2(a)). A western blot analysis with anti-TCTP antibodies recognized two species of TCTP, a 25 kDa protein and a 39 kDa protein in both the cytoplasm and nuclear fractions. Interestingly, the 39 kDa species was more abundant in the nucleus compared to the cytoplasm. To determine if TCTP is sumoylated, we first immunoprecipitated the TCTP from both cytoplasm and nuclear fractions, ran it on a gel, and probed with anti-SUMO antibodies. Our results confirm that the 39 kDa TCTPs present in the cytoplasm and nuclear fractions

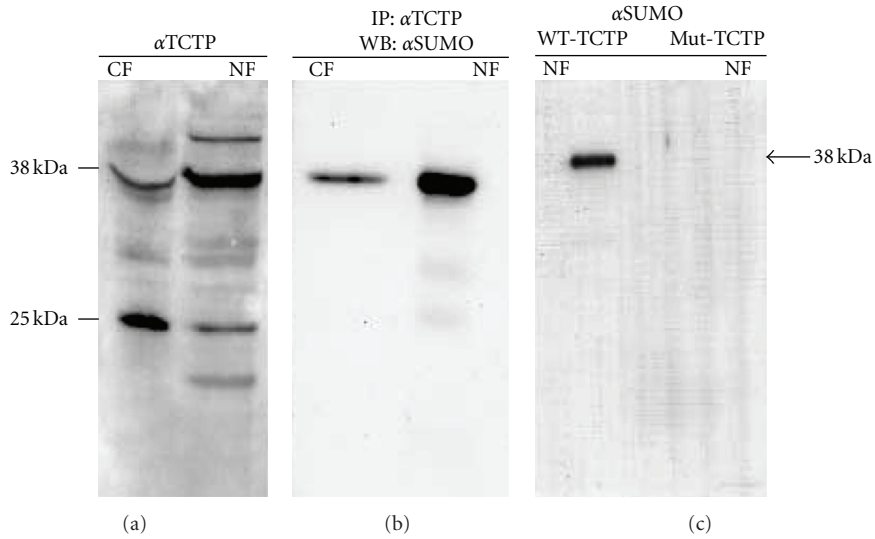


Figure 2: TCTP present in the nucleus is sumoylated. Lanes 1 and 2 show the proteins in the cytoplasm (CF) and nuclear (NF) fractions probed with an anti-TCTP antibody (a). The TCTP was then immunoprecipitated from these fractions using the anti-TCTP antibodies and the precipitate was subsequently probed with an anti-SUMO antibody (b). Lanes 3 and 4 show that sumoylated TCTP is present in both cytoplasm and nucleus. In order to determine if sumoylation is critical for the transport of TCTP into the nucleus, we used a FLAG-tagged TCTP to monitor the movement of TCTP inside the cell. Cos1 cells were transfected with either FLAG-tagged wild-type TCTP (W) or FLAG-tagged mutated TCTP (M). To determine whether the FLAG-tagged TCTPs were sumoylated, an anti-FLAG monoclonal antibody was used to immunoprecipitate the FLAG-tagged TCTP from the cytoplasm and nuclear fractions of the Cos1 cells. The bound TCTPs were then eluted, separated on a 12% gel, transferred to a nitrocellulose sheet, and probed with polyclonal anti-SUMO antibodies (c). These studies confirm that the high molecular weight form of wild-type TCTP present in the nuclear fraction is sumoylated. Results presented are representative of three similar experiments.

were sumoylated (Figure 2(b)). As expected only the high molecular weight form of TCTP was sumoylated and higher amounts of sumoylated TCTPs were present in the nucleus compared to the cytoplasm fraction (Figure 2(b)).

In the next series of experiments, we wanted to confirm that sumoylation is important for transport of TCTP into the nucleus. In these studies we used Cos1 cells transfected with FLAG-tagged TCTP. The flag helps us to identify and purify TCTP. In Figure 2(c) we show that we can demonstrate the presence of TCTP in the nuclear fraction of Cos1 cells. However, when we transfect Cos1 cells with mutated (K164R) FLAG-tagged TCTP, they fail to enter the nucleus (Figure 2(c)) and remain only in the cytoplasm. These studies confirm that sumoylation is critical for the transport of TCTP into the nucleus.

*3.5. TCTP Can Partially Rescue the Cells from $H_2O_2$ Damage.* When human granuloma cells were exposed to oxidative stress, there was a significant reduction in the viability of the cells (Figure 3). Knocking down of the TCTP within the cells with siRNA further reduced the viability of the cells compared to cells mock-transfected with Lamin (Figure 3). These results suggest that TCTP within the cells can partially rescue cells from oxidative stress.

*3.6. Overexpression of TCTP Conferred Resistance to $H_2O_2$ Damage.* Cos1 cells overexpressed with TCTP were less susceptible to the damaging effects of oxidative stress induced by

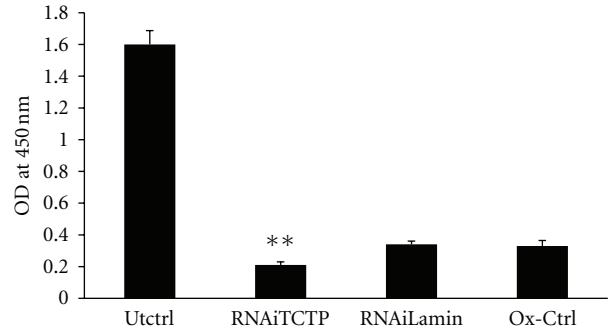


Figure 3: Knock down of TCTP by siRNA sensitizes cells to oxidative stress. Knocking down the wild-type TCTP with siRNA (RNAiTCTP) before exposing the cells to $H_2O_2$ significantly reduced the viability of human granuloma cells. Cells transfected with lamin SiRNA (RNAiLamin) or nontransfected cells exposed to the same conditions ($O_x$-Ctrl) or cells without exposure to $H_2O_2$ (Utctrl) served as controls. Data presented is average of 3 wells and is representative of three similar experiments. $^{**}P < 0.05$ compared to control siRNA (RNAiLamin) transfected group.

$H_2O_2$ (Figure 4). These results confirm our previous findings that TCTP has antioxidant function and can protect the cells from oxidative stress. To test if entry of TCTP into the nucleus is critical for its antioxidant function, we transfected Cos1 cells with TCTP mutated (K164R) in their SUMO motif (Mut-TCTP). When these cells were exposed to $H_2O_2$,

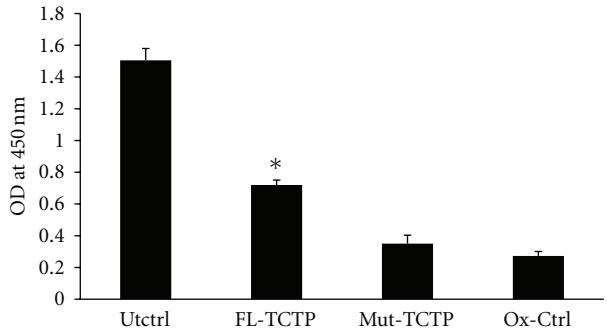


Figure 4: Over expression of TCTP conferred resistance to $H_2O_2$ damage in mammalian cells. Cos1 cells were over expressed with wild type (FL-TCTP) or mutant TCTP (Mut-TCTP) and exposed to 100 $\mu$M $H_2O_2$ for 24 h in culture. Following incubation, cell viability was determined colorimetrically. Cells transfected with vector alone and exposed to the same conditions ($O_x$-Ctrl) or without exposure to $H_2O_2$ (Utctrl) served as controls. Results show that mutation of TCTP at the SUMO motif significantly reduced its antioxidant function. $^*P < 0.01$ compared to controls.

there was a significant reduction in the viability of the cells (Figure 4). These findings thus suggest that entry of TCTP into the nucleus is critical for its antioxidant function.

## 4. Discussion

Several potential functions have been suggested for TCTP ranging from histamine release to putative antiapoptotic function. Nevertheless, a critical role for TCTP family of proteins within the cell remains to be fully described [29]. One of our recent studies showed that expression of TCTP is increased severalfold during oxidative stress and we demonstrated that TCTP can function as an antioxidant protein and as a chaperon [18, 19]. Studies by Lucibello et al. [30] show that TCTP plays a critical role in the survival of cancer cells during oxidative stress, reiterating our earlier findings that TCTP is a critical cytoprotective protein. Results presented in this study confirm that TCTP has antioxidant function. Our study also demonstrates that entry of TCTP into the nucleus is important for this antioxidant function. It is well established that TCTP is present in the nucleus. However, the mechanism of its entry into the nucleus has not been identified. In this study we show that TCTP transport into the nucleus is mediated by sumoylation.

TCTP is one of the 20 most abundantly expressed proteins within the cell [5, 6]. In fact, TCTP appears to be more abundant (approximately 100,000 copies per cell) than actin (approximately 60,000 copies/cell) in the yeast cell [31]. Majority of the TCTP proteins are present in the cytoplasm [5, 32]. However, several recent studies suggest that TCTP is also found abundantly in the nucleus, especially in certain cancer cells [13] and in cells undergoing mitosis [33] or stress [1]. It is not known how TCTP enters the nucleus. A sequence analysis of TCTP family of proteins suggested that they lack the obvious nuclear bipartite localization signals. So there has to be other mechanisms by which they enter the nucleus. Sumoylation plays a significant role in transporting proteins into the nucleus, especially proteins that lack nuclear bipartite localization signals [24, 34]. However, in order for a target protein to be transported, it should have appropriate motif for the binding of transporter protein. Analysis of the TCTP sequence using PROSITE scan tool showed that TCTPs possess potential SUMO motif with significant probability for sumoylation.

Using immunoprecipitation studies we were able to demonstrate that sumoylated form of TCTP can be found in both cytoplasm and nucleus of mammalian cells. In order for SUMO to bind to its target protein, it requires the help of an E2-conjugating enzyme, Ubc9 [25], that ligates SUMO to its target protein. The bound SUMO then covalently modifies the target protein, a process called sumoylation. In the absence of Ubc9 enzyme, the proteins cannot be sumoylated. *In vitro* functional assays in this study that measure the binding of Ubc9 and SUMO to TCTP showed that in the absence of Ubc9 enzyme TCTP was not sumoylated. Similarly, introducing a mutation in the SUMO motif (K164R) also prevented sumoylation of TCTP. Sumoylated form of TCTP is found both in cytoplasm and nucleus. When we mutated the SUMO motif in TCTP the mutated protein failed to enter the nucleus. These findings demonstrated that sumoylation of TCTP is potentially a critical event in the transport of TCTP into the nucleus. We did not do mass spectrometry or confocal microscopy to trace the movement of fluorescent-labeled TCTP from cytoplasm to nucleus. These additional techniques could have provided additional confirmatory evidence that sumoylation is critical for the transport of TCTP into the nucleus.

We previously reported that TCTP is an antioxidant protein [18] and can also function as a heat shock protein with chaperon-like activity [19]. In fact TCTP is found upregulated in various stress conditions [1] especially; the levels of TCTP are upregulated severalfold within minutes under oxidative stress [8, 15, 16]. Interestingly, SUMO and its conjugates are also increased during stress conditions [34]. Therefore, we hypothesized that sumoylation of TCTP is critical for its antioxidant function in the nucleus. Our results confirm that when we prevented TCTP from entering the nucleus by mutating SUMO motif, there was a significant reduction in the ability of TCTP to function as an antioxidant protein. The process of ubiquitylation tags the proteins for degradation, whereas sumoylation enhances their stability or modulates their subcellular compartmentalization for cell survival [27]. Thus, up regulation and entry of TCTP into the nucleus may be an important event in protecting the nuclear components and rescuing the cell from oxidative damages.

The present study thus reveals an important but hitherto unknown mechanism by which human TCTP enters the nucleus and functions as an antioxidant protein within the nucleus. Results presented in this study also suggest that sumoylation of TCTP is one such important mechanism for its nuclear transport at least in eosinophilic granuloma cells. Other redundant nuclear transport mechanism may be present for TCTP in other cell types and possibly may vary with the nature of the stress encountered. We identified one such mechanism or possibly the only mechanism of nuclear

transport of TCTP. Presence of abundant amounts of TCTP inside the nucleus suggests that this ubiquitous molecule may have potential role in protecting the DNA and the cell from oxidative stress. These studies thus reveal an important cellular function for TCTP.

## Abbreviations

TCTP: Translationally controlled tumor proteins;
SUMO: Small ubiquitin like modifier protein;
UBC9: Ubiquitin carrier protein 9.

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