Chapter 11
Elusive Role of TCTP Protein and mRNA in Cell Cycle and Cytoskeleton Regulation

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Abstract Translationally Controlled Tumor-associated Protein (TCTP) is a small, 23 kDa multifunctional and ubiquitous protein localized both in the cytoplasm and in the nucleus of eukaryotic cells. It is evolutionarily highly conserved. Certain aspects of its structure show remarkable similarities to guanine nucleotide-free chaperons Mss4 and Dss4 suggesting that at least some functions of TCTP may depend on its chaperon-like action on other proteins. Besides other functions, TCTP is clearly involved in cell cycle regulation. It is also regulated in a cell-cycle-dependent manner suggesting a reciprocal interaction between this protein and the cell cycle-regulating machinery. TCTP also interacts with the cytoskeleton, mostly with actin microfilaments (MFs) and microtubules (MTs). It regulates the cytoskeleton organization and through this action it also influences cell shape and motility. The exact role of TCTP in cell cycle and cytoskeleton regulation is certainly not fully understood. In this chapter, we summarize recent data on cell cycle and cytoskeletal aspects of TCTP regulatory role.

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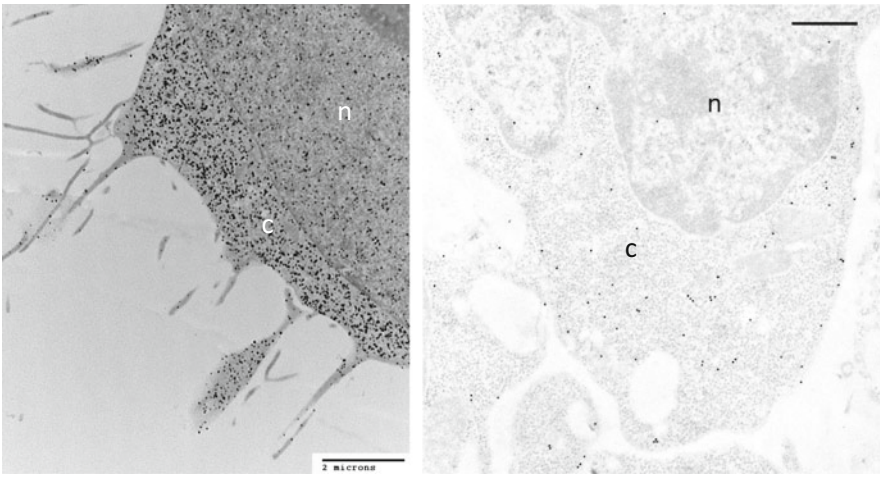

Fig. 11.1 Immunogold localization of TCTP in HIO180 cell from human ovary epithelium (*left*) and in *Xenopus laevis* ovary in oogonium (*right*). Note that TCTP is much more abundant in the cytoplasm (c) of the HIO180 cell than in the nucleus (n) and that TCTP-positive gold particles align along the actin filaments at the cell edge. In oogonium, in contrast to HIO180 cell, the nucleus (n) is almost completely devoid of TCTP

11.1 TCTP and Cell Cycle

11.1.1 The Role of TCTP Protein

TCTP is an abundant protein found in all eukaryotic cells studied so far (Bommer and Thiele 2004). It is predominantly cytoplasmic (Bazile et al. 2009), but may also localize to the nucleus (Kloc et al. 2012; Ma and Zhu 2012; Fig. 11.1). It is also a well known anti-apoptotic protein (Susini et al. 2008; Thébault et al. 2016). TCTP knockout, for instance by RNAi in human HeLa cells or *Xenopus laevis* XL2 cells, is accompanied by a rapid modification of cell shape followed by diminution of cell number in the culture because of the massive cell death (Bazile et al. 2009). The latter partially depends on TCTP capacity to downregulate p53 levels in the cells (Amson et al. 2011; Kloc et al. 2012). As p53 is also one of the major regulators of cell proliferation and cell cycle, the balance between TCTP and p53 influences these two aspects of cell physiology. However, there is an increasing body of evidences for more direct and more precise role of TCTP in cell cycle event regulation. One of them is its potential involvement in prophase checkpoint that controls the timing of M-phase entry (Burgess et al. 2008). The activation of this checkpoint via microtubule disassembly triggers transient return of early prophase cells to the G2 state (Matsusaka and Pines 2004; Mikhailov et al. 2004). One of the main actors of this checkpoint is the ubiquitin ligase Chfr (Checkpoint protein with FHA and RING finger domain). Chfr ubiquitinates Plk1 kinase and regulates the prophase checkpoint probably via Plk1 posttranslational modification since there is

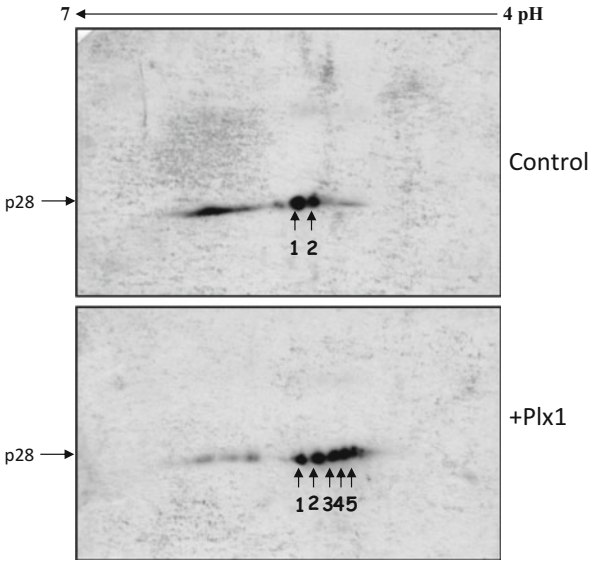

Fig. 11.2 Western blot of the 2 D gels of TCTP incubated in native conditions (control) and in the presence of active Plx1 (*Xenopus laevis* homologue of mammalian Plk1). Note the appearance of the series of new spots corresponding to novel forms of TCTP phosphorylated by Plx1

no massive Plk1 degradation upon prophase checkpoint activation (Matsusaka and Pines 2004). TCTP was shown to interact with Chfr in a microtubule-dependent manner (Burgess et al. 2008). The Chfr–TCTP interaction may be important for Chfr localization to the mitotic spindle and therefore for its proper action upon checkpoint activation. The MT disassembly provokes partial dissociation of TCTP and Chfr suggesting that the latter could be the checkpoint sensor detecting MT disassembly (Burgess et al. 2008). TCTP could play an important role by providing a link between Chfr and MTs. Despite these important data showing the involvement of TCTP in the prophase checkpoint, further analysis is necessary to fully understand its role in this process.

TCTP is phosphorylated by a cell cycle-regulating kinase Plk1 (Yarm 2002). Our unpublished results show that *Xenopus laevis* recombinant TCTP is phosphorylated by Xenopus homologue of Plk1, namely Plx1 (Bazile 2007; Fig. 11.2). This phosphorylation was proposed to decrease the microtubule-stabilizing activity of TCTP and to participate in regulation of MT dynamics during preparation for cell division and entry into the M-phase of cell cycle. As Plk1 is an important regulator of both M-phase entry (Mundt et al. 1997; Qian et al. 1999) and the mitotic spindle formation (Golsteyn et al. 1995), its kinase activity toward TCTP may allow better coordination of these processes.

The important role of TCTP in cell cycle progression and mitotic regulation was also found in human hepatocellular carcinoma (HCC) (Chan et al. 2012). The CHD1L (the chromodomain helicase/ATPase DNA-binding protein 1-like gene) is a specific oncogene of HCC. About a half of HCC cases carries CHD1L amplification. CHD1L protein directly binds to the promoter region of TCTP and activates its transcription. It has been shown that the overexpression of TCTP correlates with the increase in cell division failures and bad HCC patient prognosis.

At the molecular level, the TCTP overexpression promoted CDC25C phosphatase ubiquitin-mediated degradation by proteasomes. This in turn lowered the level of activation of the major M-phase kinase—CDK1, also known as a major enzymatic component of MPF, for M-phase Promoting Factor (Hunt 1989). CDC25C is a direct activator of CDK1 acting via dephosphorylation of two inhibitory sites on the kinase—Tyrosine 15 and Threonine 14 (Gautier et al. 1991). Therefore, proteolytic degradation of CDC25C diminishes its activation activity towards CDK1. This sequence of events results in shortening of mitosis and in consequence increases errors in chromosome separation leading to chromosome instability and an aneuploidy (Chan et al. 2012). However, it is still unclear how TCTP interacts with CDC25C phosphatase and by which mechanism it stimulates its degradation in the hepatocellular carcinoma cells. It is not clear whether TCTP acts in a similar way also in other cell types.

Knockout studies of TCTP-coding gene in the mouse has shown that in the absence of TCTP, the levels of cyclins D and E, necessary for the normal progression of the S-phase, drop dramatically resulting in lower cell proliferation (Chen et al. 2007). Thus, TCTP influences indirectly also the S-phase of the cell cycle.

11.1.2 The Role of TCTP mRNA

Several studies indicate that not only the TCTP protein, but also its transcript may play an important role in cell physiology and the cell cycle regulation. It has been shown that TCTP mRNA forms an elaborate secondary structure able to interact with protein targets. One of such proteins is protein kinase PKR, also called double-stranded RNA (dsRNA)-activated protein kinase (Bommer et al. 2002). PKR is mostly known for its involvement in viral infections. The binding of PKR to viral dsRNA triggers its autophosphorylation and activation. The phosphorylated form of PKR (pPKR) suppresses global translation via phosphorylation of the eukaryotic initiation factor 2α subunit (eIF2α). This regulation modifies multiple signaling pathways. Recent data show that among many pathways the pPKR directly regulates mitotic entry and hence the cell cycle progression (Kim et al. 2014). The pPKR is an upstream kinase for c-Jun N-terminal kinase (JNK), important regulator of the cell cycle and the cytoskeleton. It regulates the levels of mitotic cyclins and Plk1 as well as the level of histone H3 phosphorylation during mitosis. Thus, TCTP mRNA may indirectly modify cell cycle progression and cell proliferation (Fig. 11.3). Interestingly, PKR is not only activated by TCTP mRNA, but in turn it also regulates TCTP translation (Bommer et al. 2002). Thus, TCTP gene may act on the cell cycle progression using both of its gene products: the transcript and the protein. The nontranslational (structural) role of TCTP mRNA described above is a formidable example of recently described phenomenon of nontranslational roles of certain mRNAs in the organization of cytoskeleton, nucleation of subcellular organelles, and acting as a structural platform for the assembly of multiprotein complexes (Heasman et al. 2001; Jenny et al. 2006; Kanke et al. 2015; Ryu and

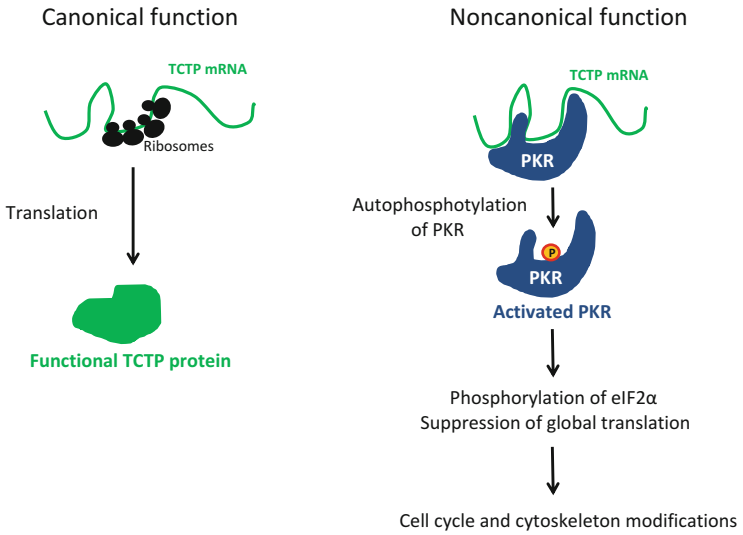

Fig. 11.3 Binary function of TCTP mRNA. *On the left*: canonical (translational) function of TCTP mRNA leading to the translation of functional TCTP protein. *On the right*: noncanonical (nontranslational) function, leading to PKR autophosphorylation and TCTP mRNA regulatory involvement in cell cycle and cytoskeleton modifications

Macdonald 2015; Blower et al. 2005, 2007; Kloc et al. 2007, 2011a, b; Kloc 2008, 2009; Kloc and Kubiak 2016, 2017).

So far, TCTP was not clearly linked to a precise cell cycle-regulating pathway. It does not seem to play a major role in a particular step of cell cycle regulation. However, on the basis of the above examples, it is very tempting to speculate that TCTP may act as a chaperon for various cell cycle regulators. Upon TCTP overexpression and in appropriate conditions, like those taking place, for example, in hepatocellular carcinoma cells, its modulatory role may be affected to such a degree that it profoundly modifies the cell cycle.

11.2 TCTP and Cytoskeleton

Although the association of TCTP with microtubules (MTs) and microfilaments (MFs) is still not very well characterized, it seems that TCTP has relatively low affinity for MTs, and much higher for MFs.

11.2.1 TCTP and Microtubules

TCTP clearly localizes to the mitotic spindle and to cytoplasmic MTs (Gachet et al. 1999; Yarm 2002; Burgess et al. 2008; Bazile et al. 2009; Jeon et al. 2016). Although TCTP is believed to interact with tubulin and MTs (Gachet et al. 1999) the TCTP pull-down experiments with purified MTs have shown very low affinity of TCTP for microtubules (Bazile et al. 2009). Similar experiments with actin MFs have shown that TCTP has high affinity for MFs when the experiment is done in the presence of cell-free extract from *Xenopus laevis* embryos (ibid.). This suggests that the cell-free extract provides either intermediate proteins that attach TCTP to F-actin or it modifies TCTP or actin in a posttranslational manner (e.g., via phosphorylation/dephosphorylation) allowing their mutual interaction. Precise localization of TCTP and tubulin in human HeLa cells and *Xenopus laevis* XL2 cells has shown that some fibers (thick filaments) positive for TCTP do not stain with anti-tubulin antibody and vice versa (Bazile et al. 2009). This suggests that TCTP may decorate other intracellular fibers than MTs. Also a co-injection of anti-TCTP antibody with rhodamine-tubulin into cells has shown that the tubulin-positive network does not entirely correspond to the TCTP-positive array (Bazile 2007; Fig. 11.4). It is still not clear what fibers are decorated by TCTP. Our electron microscopy studies using immune-gold method have shown at the ultrastructural level that TCTP localizes in the vicinity of MTs, but does not decorate them directly (Jaglarz et al. 2012). This again supports a hypothesis that the association of TCTP with MTs is indirect and more elusive than with classical MT-Associated Proteins. Moreover, we found TCTP localized in centrosomes (Bazile et al. 2009; Jaglarz et al. 2012) and more precisely, at their outer part, outside of the gamma-tubulin-containing part of the centrosome (Jaglarz et al. 2012). These data strongly suggest that TCTP is associated with MTs, but in a special way and in addition it may influence MT cytoskeleton organization via modification of certain functions of centrosomes.

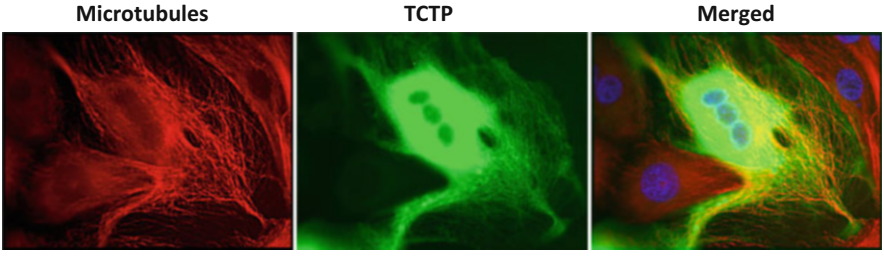

Fig. 11.4 TCTP-positive network does not exactly correspond to MT arrays. Non-overlapping of MTs (*left*; *red*) and TCTP-positive filaments (*middle*; *green*) in a cell co-injected with rhodamine-tubulin and anti-TCTP (subsequently stained green with FITC conjugated secondary antibody). The merged image of *red* and *green* is shown in the *right* panel. Nuclei (*blue*) are stained with Dapi

11.2.2 TCTP and Actin

TCTP co-localizes clearly with curly actin filaments at the cell border (Bazile et al. 2009). TCTP localization on actin filaments can be also seen in Fig. 11.1 where TCTP-positive gold particles align along the actin filaments at the border of HIO180 cell. TCTP has an actin-binding site with high homology to ADF/cofilin (Tsarova et al. 2010). This may suggest that TCTP and cofilin compete for actin binding and this competition influence actin stability. However, TCTP peptide, which is homologue to cofilin, has a higher affinity for G-actin than F-actin and does not block actin-filament depolymerization by cofilin (ibid.). Thus, TCTP seems to favor the sequestration of G-actin and in consequence targets more cofilin to F-actin. Inversely, the loss of TCTP could result in increased sequestration of cofilin by monomeric actin. Indeed, TCTP downregulation results in profound alteration of actin cytoskeleton (Liu et al. 2015). At least a part of this action may depend on the competition between TCTP and cofilin for G-actin-binding sites.

Finally, TCTP is a calcium-binding protein (Kim et al. 2000; Arcuri et al. 2004) and thus may participate in the cytoskeleton organization through local modifications of calcium homeostasis in the cytoplasm. Interestingly, it is known that actin-filament polymerization depends on calcium ions and that any disturbance in calcium homeostasis may influence actin-dependent cell shape (Ishida et al. 2017). Thus, it will be very interesting to study TCTP and actin interactions during cell shape changes related to cell polarization, cell movement, and interaction with extracellular matrix and neighboring cells.

11.3 Conclusions

TCTP plays multiple roles in cell physiology. It is a pro-proliferative and anti-apoptotic protein. Besides its direct role in the negative regulation of apoptosis, it participates in cell cycle regulation. It is involved in prophase checkpoint and in the control of M-phase duration. Its association with microtubules, actin, and centrosomes suggests that it participates in the coordination of the organization of the cytoskeleton. Its calcium-binding properties suggest that it may regulate calcium homeostasis and actin-related functions in the cell. In addition, potential structural (nontranslational functions) of TCTP mRNA should be an extremely interesting subject for future studies.

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