Cellular Function and Regulation of the Translationally Controlled Tumour Protein TCTP

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Abstract: The 'translationally controlled tumour protein' TCTP was originally discovered 30 years ago by researchers interested in proteins regulated at the translational level. Cloning and sequencing confirmed the conservation of this protein among all eukaryotic kingdoms, but did not reveal any functional clue, and TCTP was listed in the databases as a 'family' of its own. The functional characterisation of this protein extended over more than a decade, leading to a plethora of individual functions and interactions that have been ascribed to this protein. A major addition to the functional characterisation of TCTP was the identification in 1995 of its histamine releasing factor (HRF) activity in allergic conditions, which for the first time described an extracellular activity for TCTP in human disease. This triggered a host of additional publications aimed at characterising this HRF activity, which are discussed in other articles of this issue. Another milestone in the elucidation of TCTP's function was the demonstration of its anti-apoptotic activity in 2001. Evidence is also accumulating for a role of TCTP in the cell cycle and in early development. This article provides an overview of the main cellular activities of TCTP. The second part will summarise our current knowledge on the mechanisms involved in regulating intracellular TCTP levels.

Keywords: TCTP, histamine releasing factor, fortilin, mitotic regulation, anti-apoptotic protein, mTORC1 signalling, tumour protein, translational regulation.

INTRODUCTION

1. The Translationally Controlled Tumour Protein TCTP - History of Discovery

The first reports on the translationally controlled tumour protein TCTP date back about 30 years. The protein was discovered independently by three groups studying proteins that are regulated at the translational level of gene expression. George Thomas investigated proteins that are regulated in response to mitogenic stimulation of mouse fibroblasts and identified a translationally controlled protein 'Q23' [1]. The group of George Brawerman (Boston) studied mRNAs that are abundantly represented in untranslated, non-ribosomal mRNP particles, and among these identified the mRNA for a protein they called 'P21' [2]. In Heinz Bielka's group (Berlin) the protein 'P23' was found to be preferentially synthesized in exponentially growing vs. serum-starved Ehrlich ascites tumour cells [3]. The first cDNA sequences of the mouse [4] and human [5] protein where published around this time, and in the latter paper, the protein was termed 'translationally controlled tumour protein', since it was cloned from a human mammary tumour. The additional designations 'histamine releasing factor, HRF' [6] and 'fortilin' [7] were proposed later by the groups who discovered the histamine release and anti-apoptotic activities of TCTP, respectively. However, since these terms relate only to a specific activity of this multifunctional protein, the older, more unspecific name TCTP is still being used at large.

2. Molecular Structure and Conservation of TCTP

The cloning and sequencing did not provide any clue about the functional importance of TCTP, since no similarity to other proteins or functional domains was discovered, and thus the protein was listed as a 'family' on its own in the databases. As more and more TCTP sequences were deposited in the database, it became clear that TCTP is highly conserved among all eukaryotic kingdoms (reviewed in [8]). For a recent detailed sequence comparison and phylogenetic analysis see Hinojosa-Moya *et al.* [9].

Elucidation of the first 3D structure of the TCTP from the fission yeast *S. pombe* [10] demonstrated that the TCTP molecule consists of three distinct domains, the core β-sheet domain, an α-helical domain and a flexible loop structure (Fig. **1**). Many additional TCTP structures subsequently deposited in databases, confirmed that this principal structure is highly conserved in evolution. The first NMR structure analysis also revealed the similarity of the highly conserved β-sheet domain of TCTP with Mss4/Dss4 proteins, which bind to the GDP/GTP-free form of Rab proteins [10]. This discovery indicated that TCTP might be related to GTPase-associated proteins and two examples of such functional activity will be discussed below.

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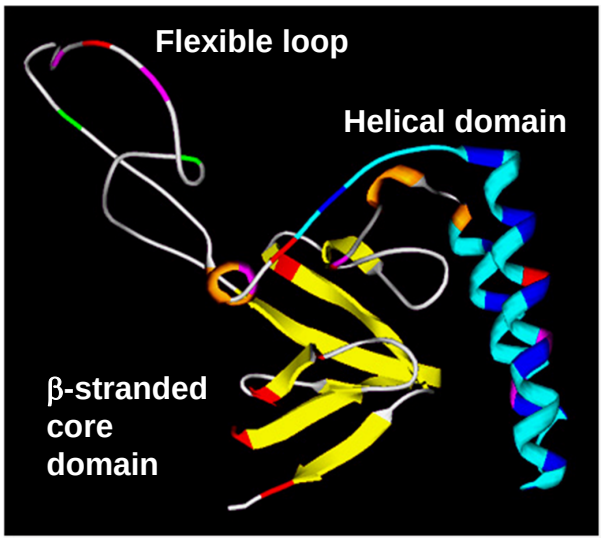

Fig. (1). Three-dimensional structure of the TCTP protein. The ribbon structure of fission yeast TCTP as originally published by Thaw *et al.* [10] is shown (PDB: 1H6Q). The domain structure is indicated. The β-stranded core domain is shown in yellow, and the α-helical domain region is marked in blue. The most conserved residues are labelled in red or magenta. Green indicates the positions of the serine residues phosphorylated by the mitotic kinase Plk-1 [19].

This is the only indication of a functional activity, which arose from structural studies of TCTP. However, a plethora of additional functional activities has been described since by numerous research groups. It is the aim of this article to provide a current overview on the many molecular associations and cellular functions of TCTP, as well as on the mechanisms involved in the regulation of cellular TCTP levels.

CELLULAR FUNCTIONS OF TCTP

1. Interaction of TCTP with the Cytoskeleton and its Role in Cell Division

- ***TCTP – A Non-Canonical Ca^{++}-Binding Protein***

One of the earliest demonstrations on a functional activity for TCTP was the report on the Ca^{++}-binding activity of trypanosome TCTP by Haghigat & Ruben [11]. Since then, many groups have demonstrated Ca^{++}-binding of TCTP by means of the Ca^{++}-overlay assay (see e.g. [12]), even though the TCTP sequence does not harbor a canonical Ca^{++}-binding motif.

In 2007, two detailed studies have been published aimed at characterising the Ca^{++}-binding site in TCTP. Feng and co-workers [13] used multi-dimensional NMR spectroscopy to determine the solution structure of human TCTP and to identify the Ca^{++}-binding site. They concluded that the weak binding site involves the residues N131, Q132 and D150, located in the β-stranded core domain close to the connection with the α-helical domain. In contrast, Graidist *et al.* [12] employed a combination of several methods to study the Ca^{++}-binding activity of TCTP. They performed a detailed mutational analysis, leading them to conclude that the residues E58 and E60, located in the floppy loop of the TCTP structure, are involved in Ca^{++}-binding. These two apparently differing results might be reconciled, if there is more than one binding site for Ca^{++} in the TCTP molecule. However, more studies will be necessary to resolve this question.

- ***Interaction with the Cytoskeleton***

The first intracellular function described for TCTP, i.e. microtubule binding and stabilisation, was unveiled by our laboratory [14]. We demonstrated that TCTP (P23) associates with microtubules in a cell cycle-dependent manner. We identified the tubulin binding domain of TCTP, which is largely identical with the α-helical domain of the 3D structure (Fig. **1**). Overexpression of TCTP resulted in growth retardation of cells, microtubule stabilisation and alteration of cell morphology.

A recent, more detailed study into the interaction of TCTP with the cytoskeleton revealed that TCTP also interacts with actin filaments; it localises to a subset of actin-rich fibers in migrating cells [15]. This observation is corroborated by another report on a domain in TCTP, similar to the actin-binding domain in cofilin [16]. Bazile *et al.* [15] also demonstrated that TCTP's interaction with the microtubules is transient, and that it does not behave as a *bona-fide* microtubule-associated protein.

- ***Binding to the Mitotic Spindle and Mitotic Phosphorylation of TCTP***

In our study we reported that TCTP (P23) is bound to the mitotic spindle, but is detached from the spindle during metaphase-anaphase transition [14]. TCTP binding to the spindle, predominantly to the poles, has since been confirmed by reports from other laboratories [17,18]. It is anticipated that TCTP is involved in stabilising spindle microtubules [17,19], and the observed detachment of TCTP from the spindle might be important for the metaphase-anaphase transition. We also observed that TCTP is subject to mitotic phosphorylation (M. Lee, Y. Gachet and U.A. Bommer, unpublished results), and we anticipated that this phosphorylation event is necessary for the detachment of TCTP from the spindle microtubules. The protein kinase involved in TCTP phosphorylation was subsequently characterised by Yarm [19] as the mitotic polo-like kinase Plk-1. He also identified two phosphorylation sites for Plk-1 in the flexible loop of the TCTP structure (Fig. **1**). Expression of a TCTP protein mutated in these sites led to severe disturbance of mitotic progression and to the formation of multinucleated cells. The phosphorylation of TCTP by Plk-1 has since been studied by another laboratory [20], and it has been proposed as a marker for the action of anti-cancer drugs, targeting Plk-1 [21].

- ***TCTP as a General Mitotic Regulator***

There is an increasing body of evidence indicating that TCTP is indeed an important mitotic regulator, and mechanisms other than stabilisation of spindle microtubules have been proposed to play a role in this activity. A range of nuclear proteins involved in mitotic progression have been reported to interact with TCTP, and either to regulate TCTP levels or being regulated by TCTP.

Chfr is a checkpoint protein that plays an important role in cell cycle progression. Burgess *et al.* [17] demonstrated that lowly expressed ectopic Chfr is localised in the cytoplasm, but localises to the spindle during mitosis. They also

showed that Chfr interacts with TCTP throughout the cell cycle, unless cells are treated with microtubule-destabilising agents. Two separate reports by Johansson *et al.* [22, 23] describe the interaction of TCTP with the two nuclear proteins, nucleophosmin and nucleolin, in embryonic stem cells. In the case of nucleophosmin, the interaction was shown to be independent of phosphorylation by Plk-1.

A recent paper unveiled a novel pathway, through which TCTP is likely to be involved in the dysregulation of mitotic progression in hepatocellular cancer, HCC [24]. The authors identified the TCTP gene, tpt1, as a target for transcriptional activation by CHD1L, a specific oncogene frequently upregulated in HCC. Overexpression of TCTP promoted the ubiquitin-proteasome degradation of the phosphatase Cdc25C. During mitotic progression, Cdc25C is essential for the final activation of Cdk1. The drop of Cdk1 activity induced by TCTP overexpression resulted in a too fast mitotic exit and consequently led to chromosome miss-segregation. The effect of TCTP overexpression on mitotic progression in tumour cells described here are consistent with earlier papers reporting that over- or underexpression of TCTP affects the duration of the cell cycle [14, 23, 25]. The observation that TCTP is one of the proteins ubiquitinylated and partially degraded in proteasomes during mitosis [18] would also indicate that tight regulation of TCTP levels is essential for the orderly exit of cells from mitosis.

It is well documented from plants [25, 26], as well as from lower animals, such as cnidarians [27], that TCTP is preferentially expressed in meristmatic/proliferative active tissues, and recently, a thorough study described TCTP as a general mitotic regulator in plants and animals [25]. This group demonstrated that TCTPs are interchangeable between plants (*Arabidopsis*) and animals (*Drosophila*) in their ability to rescue mitotic defects in the other species.

- ***Importance of TCTP in Early Development***

The involvement of TCTP in mitotic regulation indicates that it could play a role in the early development of animals and plants. There are several lines of evidence clearly supporting this view:

1. Complete gene knockout of TCTP in Drosophila [28] and in mice [29-31] is embryonic lethal, and this effect was related to excessive apoptosis during early embryonic development of these mice. Koide *et al.* [31] provided data showing that TCTP functions as an inhibitor of the BMP pathway in the early phases of development.
2. In 2007, two papers reported a role for TCTP in the reprogramming of somatic cell nuclei to an embryo-like gene expression pattern after transplantation into oocytes [32,33]. In this process, the transcription factor oct4, which is also considered a stem cell marker, is massively up-regulated. Koziol *et al.* [32] identified TCTP as one of the proteins that bind to the regulatory region of the mouse oct4 gene and that activates its expression and hence the activation of genes involved in pluripotency. As mentioned in the previous section, TCTP was described as interaction partner for nucleolin [22]. This paper also demonstrated that nucleolin interacts with the transcription factor oct4 in human and murine embryonic stem cells.
3. Several reports indicate that TCTP plays a crucial role in early gametogenesis. Guillaume *et al.* [34] found high expression levels of TCTP in most stages of spermatogenesis, and Vitale *et al.* [35] showed that TCTP expression is altered during murine oocyte maturation. Meyvis and coworkers [36] reported that knock-down of TCTP in *C. elegans* reduced the numbers of eggs laid by the hermaphrodite in the F(0) and F(1) generations, indicating a pivotal role of TCTP in egg production.
4. A critical process in early mammalian development is the implantation of the fertilised egg. A recent publication reports that TCTP mRNA and protein levels increase in the uterus of mice during early pregnancy, reaching a peak at D5, but then decrease dramatically to D7 [37]. The peak of TCTP expression coincided with the implantation phase, and at this time TCTP levels were considerably lower in the uteri of pseudo-pregnant control mice. The number of implanted embryos was reduced when TCTP levels were down-regulated by injection of antisense oligos. Arcuri *et al.* [38] described a role for TCTP in Ca^{++}-handling and transport in the placenta. TCTP knock-down was associated with reduced cellular calcium-uptake and buffering capacity.
5. In plants, knockout of TCTP leads to a male gametophytic phenotype, with normal pollen formation and germination, but impaired pollen tube growth [26]. Silencing of TCTP by RNA interference slowed vegetative growth, and leaf expansion was reduced because of a smaller cell size. These data indicate that TCTP is also important for plant cell growth and development.

2. Part of the Cell's Survival Kit: The Anti-Apoptotic Protein TCTP

- ***Protection Against Diverse Cell Stresses***

TCTP was originally described as a growth-induced protein (see section on TCTP and growth regulation below), and later it became clear that it is also highly regulated in response to a range of additional cellular stimuli and stress conditions. Examples of stress-dependent regulation of TCTP levels will be detailed in the regulation section below. Here, I will summarise the various mechanisms in discussion to-date, by which TCTP may exert its cyto-protective function.

- ***A Role for TCTP in Ion-Homeostasis: Regulation of the Na^+-K^+-ATPase***

A potential role of TCTP in the regulation cellular ion homeostasis was revealed by Kyunglim Lee's group in Seoul. Using a yeast two-hybrid screen, they established that TCTP interacts with the 3rd large cytoplasmic domain of two isoforms of the Na^+-K^+-ATPase [39]. They also showed that overexpression of TCTP resulted in inhibition of the enzyme. The Na^+-K^+-ATPase had been implicated in the pathogenesis of hypertension, and the authors hypothesised that overexpression of TCTP might promote the development of hypertension. They generated TCTP overexpressing

mice, which indeed developed systemic arterial hypertension, about six weeks after birth [40].

- ***Anti-Apoptotic Properties of TCTP (Fortilin)***

The first demonstration of the anti-apoptotic function of TCTP came from Fujise's laboratory in Houston [7]. Importantly, these authors demonstrated that overexpression of TCTP protected HeLa cells from undergoing etoposide-induced apoptosis. Based on their observations, they coined the name 'fortilin' for this protein and since refer to it under this name. Anti-apoptotic activity of TCTP has since been reported in numerous papers and for a range of cellular conditions. Here, I will give a few of the recently published examples:

Gnanasekar *et al.* [41] showed that human TCTP and its homologue from the parasite *Schistosoma mansoni* may act as a molecular chaperone under heat shock conditions, protecting other proteins against thermal denaturation. Several groups reported regulation of TCTP levels under Ca^{++}-stress conditions [42-44], and overexpression of TCTP partially protected cells from induction of apoptosis by Ca^{++}-stress [12,42]. The importance of TCTP as a survival factor, involved in the development of cellular resistance against anti-cancer drugs was demonstrated earlier for etoposide [7] and 5-fluorouracil [45].

The role of TCTP in protecting cells against oxidative stress was demonstrated through cloning of genes that confer such protection to NIH-3T3 cells [46]. Gnanasekar *et al.* [47] reported that TCTP of the parasite *Brugia malayi* functions as an antioxidant protein. More recently, Lucibello *et al.* [48] studied the importance of TCTP in the protection of cancer cells against oxidative stress, and showed that it is an important survival protein likely to be involved in the resistance against anti-cancer therapy. Interestingly, this paper also reports that glucose deprivation resulted in down-regulation of TCTP, followed by cell death. The latter finding is corroborated by our recent study on pancreatic beta-cells, showing that an exposure to high glucose (25 mM) leads to an increase in intracellular TCTP levels [43]. This paper also demonstrated that overexpression of TCTP partially protects beta-cells against the induction of apoptosis by high levels of saturated fatty acids (palmitate), a particular stress situation for these cells.

The role of TCTP as an anti-apoptotic protein was highlighted in TCTP gene-knockout studies on mice [29-31], which demonstrated embryonic lethality due to excessive apoptosis at an early embryonic state. In 2009 two papers showed the importance of TCTP in preventing apoptotic cell death for the maintenance and development of T-cells. Using conditional TCTP knockout mouse models, Wu and colleagues [49] demonstrated that TCTP is essential for the viability of mature peripheral T-cells. Xiong *et al.* [50] reported that IL-2 promotes the survival of regulatory T-cells through stimulation of TCTP expression.

- ***Mechanisms of Anti-Apoptotic Activity***

TCTP has been shown to interact with two other anti-apoptotic proteins of the Bcl-2 family, i.e. Mcl-1 [51, 52] and Bcl-XL [53]. Bcl-XL and TCTP have both been implicated in maintaining the survival of murine T-cells during activation [53]. Two reports indicate that Mcl-1 and TCTP stabilise each other, with TCTP being stabilised by Mcl-1 [52] but also vice versa [51]. Yet another paper showed that they exert their anti-apoptotic function independent of each other [45].

A range of mechanisms have been proposed as to how TCTP exerts its anti-apoptotic activity. Graidist *et al.* [12] demonstrated a role of TCTP as a Ca^{++}-scavenger, preventing excessive increase in intracellular Ca^{++}-levels in Ca^{++}-stress conditions. Gnanasekar and colleagues reported that TCTP acts as molecular chaperone under heat shock conditions [41]. Telerman's group [30] proposed a novel mechanism, according to which TCTP, with its helical domain, inserts itself into the mitochondrial membrane, thereby antagonising the dimerisation of the pro-apoptotic protein Bax and preventing the Bax-induced increase of mitochondrial membrane permeability. A cell stress-dependent association of TCTP with mitochondria has been previously observed in yeast [54].

A peculiar involvement of TCTP in anti-apoptotic intercellular signalling was recently proposed by Sirois and coworkers [55]. This paper reports that TCTP is one of the marker proteins present at the surface of the nanovesicles released from apoptotic endothelial cells. These nanovesicles, which are different from apoptotic blebs, induce an anti-apoptotic phenotype in vascular smooth muscle cells (VSMC). Pre-treatment of endothelial cells with TCTP siRNAs attenuated the anti-apoptotic activity of purified nanovesicles on VSMCs.

- ***Antagonism Between TCTP and P53***

Antagonism to the tumour suppressor protein p53 has recently been proposed as an another mechanism by which TCTP can prevent apoptotic cell death. It is not surprising, that activation of p53, one of the most powerful pro-apoptotic proteins, down-regulates the anti-apoptotic protein TCTP. Such observations have been made earlier, using a temperature sensitive mutant of p53, in two quite different cellular systems [42,56]. Several recent studies now describe TCTP as an interaction partner and antagonist for p53, albeit by differing mechanisms. Rho *et al.* [57] showed that overexpression of TCTP in lung carcinoma cells reversed p53-mediated apoptosis, whereas TCTP knock-down increased apoptosis. Similarly, Chen and colleagues [58] observed TCTP (fortilin) interaction with p53 using GST-pull down assays; they showed that TCTP inhibits p53-dependent apoptosis. However, these two studies arrive at differing conclusions in the following two points: 1. In the mapping of the domain in TCTP that is involved in p53 binding: Rho *et al.* [57] found that an internal domain (corresponding to the α-helical domain; Fig. **1**) is involved in p53 binding, whereas Chen *et al.* [58] observed that the N- and C-terminal parts of the molecule (the β-sheet domain; Fig. **1**) participate in this interaction. 2. In the mechanism by which TCTP antagonises p53: Rho *et al.* reported that TCTP is destabilising p53 in lung cancer cells, whereas Chen and colleagues did not observe p53 destabilisation, but instead proposed that TCTP blocks p53-induced transcriptional activation of Bax [58].

A profound study on this issue has just been published by the Telerman group [59]. They describe details of a negative

feedback loop between P53 and TCTP, according to which TCTP promotes MDM2-mediated ubiquitination and degradation of P53. Conversely, P53 directly represses TCTP transcription. This paper also provides evidence for the implication of this antagonism to P53 in cancer, which will be discussed below.

3. Involvement of TCTP with Growth Regulation and the Translational Machinery

• *TCTP and Cell Growth Regulation*

Translational induction of TCTP synthesis upon growth stimulation of mammalian cells was one of the earliest observations about this protein [1, 3]. We typically observed an about four-fold increase of TCTP levels after growth induction of mouse fibroblasts [60]. Growth-dependent regulation of TCTP synthesis has been observed by many groups, as reviewed in our earlier article [8]. In the last decade, an overwhelming body of evidence has associated TCTP with cancer, as will be discussed below. A detailed study on TCTP in *Arabidopsis thaliana* demonstrated that knockdown of this protein generated serious growth defects [26], highlighting the importance of TCTP as a growth regulator, also in plants.

All these observations reflect a positive association with cell growth, however the question remains unanswered: What is the underlying mechanism, by which TCTP positively affects cell growth? It is attractive to speculate that TCTP's involvement in mitotic regulation might be important. However, we observed that overexpression of TCTP actually slows cell cycle progression [14], and very recently Chan *et al.* [24] showed that increased TCTP induces mitotic defects and chromosome miss-segregation in hepatocellular carcinoma. Thus, it appears that controlled expression of TCTP is essential for an orderly transition through the cell cycle, making it unlikely that mass induction of TCTP levels are required for progression through mitosis.

Two studies reported that an increase in TCTP levels results in activation of cell growth signalling pathways: Kim *et al.* [61] found that in HeLa cells, TCTP overexpression resulted in activation of several growth signalling pathways, inclusive of tyrosine phosphorylation of the EGF receptor, phosphorylation of PLC-γ, as well as activation of the Ras/Raf/ERK and the PI3K/Akt pathways. Specific inhibition of the PI3K/Akt pathway significantly decreased TCTP overexpression-induced cell survival, whereas inhibition of PLC-γ pathway diminished TCTP overexpression-induced cell migration. In a more recent study, the same group performed similar experiments on human breast epithelial cells [62]. They found that TCTP induces release of the protein kinase Src from the Na^+-K^+-ATPase. Src activation then resulted in tyrosine phosphorylation of epidermal growth factor receptor and the activation of a range of signalling pathways, such as the PI3K/Akt, the MAP kinase, and the PLC-γ pathways, apart from other cellular alterations. Conversely, Wang and Dao [63] reported that TCTP is down-regulated during neural differentiation of mouse embryonic stem cells.

The regenerating rat liver after partial hepatecomy is a classical model for tissue growth. Zhu *et al*. [64] studied TCTP mRNA expression in this model and demonstrated that TCTP mRNA levels were significantly up-regulated in the period from 3 to 12 hours after partial hepatectomy, but then decreased at 24 hours before returning to original levels.

Cell growth is intimately linked with an increase in protein synthesis, and early observations demonstrated that both protein synthesis and ribosome biogenesis are largely increased after partial hepatectomy. Several other observations also potentially link TCTP with the translational machinery and its regulation; these will be discussed in the following section.

• *Is TCTP an Upstream Regulator of the mTOR Signalling Pathway?*

The mammalian target of rapamycin complex 1 (mTORC1) signalling pathway is a major regulator of protein synthesis in mammalian cells [65]. This pathway represents a complex signalling network, which integrates a range of positive and negative input signals and positively regulates protein synthesis, as well as other anabolic pathways. Most relevant for the regulation of protein synthesis is the regulation of mTORC1 in response to growth factor signalling, to amino acid availability and to the energy status of the cell.

An important upstream activator of mTORC1 is the small GTPase Rheb (Ras homologue enriched in brain), which in turn is negatively regulated by its GTPase-activating protein, the tumour suppressor protein TSC1/TSC2. The guanosine nucleotide exchange factor (GEF) for Rheb remained for long time (and still is) enigmatic. In 2007, Hsu *et al.* [28] published a paper describing TCTP as a potential GEF for Rheb. They presented data indicating that reducing *Drosophila* TCTP (dTCTP) levels reduced cell size, cell number and organ size, resembling dRheb mutant phenotypes. They also found that dTCTP directly associates with dRheb and can act as a GEF for Rheb. Thus, this paper ascribed an important role to TCTP, as a positive regulator of the TOR growth signalling pathway.

The idea that TCTP acts as a GEF for small GTPases, such as Rheb, appeared attractive based on TCTP's structural similarity to MSS4/DSS4 proteins, which are known to bind to the Rab family of GTPases [10]. However, subsequent investigations aimed at confirming TCTP's importance in regulating mTORC1 signalling in mammalian cells failed to support the findings published for *Drosophila* TCTP: We observed that reducing TCTP levels did not reproducibly affect mTORC1 signalling in HEK 293 cells [66]. Moreover, overexpression of TCTP did not rescue mTORC1 signalling in amino acid-starved cells, and no stable interaction between TCTP and Rheb or mTORC1 was observed. Similarly, Rehmann *et al.* [67] were unable to detect GDP exchange activity of TCTP towards Rheb. TCTP depletion in cells did not affect direct downstream targets of Rheb, and no interaction between TCTP and Rheb could be detected by NMR spectroscopy. However, another study reported a weak binding between Rheb and TCTP, and also a GEF activity for TCTP towards Rheb [68]. These authors also performed further structure modelling in support of the interaction. – Overall, this issue is still controversial. We are aware, from personal communications, that other laboratories also obtained negative results in this regard, which have not been

Table 1. TCTP Expression in Cancer Cell Lines

Cells Derived From	Cell Lines	Result Regarding TCTP	Reference
Colon cancer	SNU-C4, C5	mRNA levels 2 - 4.5 fold increased compared to normal cells	[109]
Colon cancer	LoVo-cells	TCTP knock-down results in decreased cell proliferation, migration, invasion and tumorigenicity.	[81]
Prostate cancer	LNCaP	TCTP knock-down results in decreased cell viability and increased apoptosis	[79]
Bronchial epithelia (transformed)	1170-I, 1198,	TCTP secretion is two-fold higher in transformed *vs.* non-transformed cells.	[75]
(non-transformed)	BEAS-2B, 1799		
Human leukaemia	U937, K562	Reduction of TCTP levels leads to reduced tumorigenicity in mice and reversion from the malignant phenotype.	[56]
Breast carcinoma	MDA-MB231S BT20, T47D		
Colon cancer Lung cancer Melanoma	DLD-1 A549 various lines	Cell clones reverted from the transformed to the normal phenotype display lower TCTP expression.	[77]
Multiple Myeloma	RPMI8226		[78]

published. Therefore, currently this potentially interesting function of TCTP remains an unresolved question.

- ***Is TCTP Part of the Translational Apparatus and a Downstream Target of mTOR?***

The mRNA for TCTP starts with a 5'-terminal oligopyrimidine tract (5'-TOP) [69]. This observation suggests that TCTP expression may be subject to translational control via the mTORC1 signalling pathway. Almost all mRNAs that bear this signature encode proteins that are part of the translational apparatus, and they are known to be translationally up-regulated through mTORC1 [65]. It therefore seems likely that TCTP mRNA is a downstream target of mTOR (see TCTP regulation; below).

Direct evidence for a role of TCTP in the translational machinery came from a study by Cans *et al.* [70]. Using a yeast two-hybrid search, they identified TCTP as a binding partner for protein synthesis elongation factor eEF1A, and its guanine nucleotide exchange factor, eEF1Bβ. The interaction of TCTP with eEF1Bβ was also confirmed by Langdon *et al.* [71]. Cans and colleagues demonstrated that TCTP preferentially stabilizes the inactive GDP-bound form of eEF1A, by impairing the GDP exchange reaction promoted by eEF1Bβ. From this study, it would appear that TCTP acts as a negative regulator for eEF1A, which is apparently inconsistent with a growth-promoting role of TCTP.

A more recent report provided an interesting twist to this story: Leclerq *et al.* [72] performed a study on Sphingosine kinase 1 (SK1), which catalyses the formation of the phospholipid sphingosine 1-phosphate. Elevated activity of this enzyme enhances cell proliferation and survival, and is implicated in tumorigenesis. This group had previously shown that eEF1A interacts with and activates SK1. In this study, they demonstrate that it is [eEF1A x GDP], but not [eEF1A x GTP], that activates SK1 activity *in vitro*. They also show that enhancing cellular [eEF1 x GDP] levels through expression of TCTP (which acts here as guanine nucleotide dissociation inhibitor of eEF1A) increases cellular SK1 activity. In this way, TCTP could be implicated in tumorigenesis, even though being a negative effector of protein synthesis. More work is required to determine, which potential role TCTP might play in protein synthesis.

4. Is TCTP a Tumour Protein?

- ***TCTP Expression in Human Tumours***

The observation that TCTP is expressed in nearly all cell types and tissues, albeit at differing levels [73] initially raised some doubts on the justification of the term 'tumour protein' [74]. However, over the past decade, a substantial body of evidence has accumulated, demonstrating that TCTP is positively related to cancer: First, several studies on cancer cell lines, derived from a range of cancer types, have shown that TCTP levels are positively related to properties of these cells relating to growth behaviour, anti-apoptotic properties and tumorigenicity (Table **1**). Second, studies on several human cancers have demonstrated that TCTP levels are up-regulated in most, but not all, of the investigated cancer types (Table **2**). In some cases, such as lung cancer [75], hepatocellular carcinoma [24] and breast cancer [59], TCTP has even been proposed to be suitable as a tumour marker. Our recent data obtained by immunohistostaining of human colon cancer samples also indicate that TCTP levels are significantly increased in adenomas and adenocarcinomas of the colon, compared to normal colon tissue (M. Radojkovic, P. Colligan, P. Puri, A. Lochhead, M. Aghmesheh & U. Bommer, unpublished results).

- ***TCTP in the Tumour Reversion Model***

The importance of TCTP in cancer has been convincingly demonstrated through studies using the tumour reversion model. This model is based on the rare event of tumour cells reverting back from the malignant to the normal phenotype (reviewed in [76]). Telerman's group studied this system intensively and identified a number of genes that are particularly involved in this process, of which TCTP appears to play a major role. In particular, they showed that inhibition of TCTP expression by siRNA results in suppression of the malignant phenotype [56]. They also demonstrated: (1.) that revertants derived from melanoma cell lines, colon or lung cancer show decreased TCTP levels; (2.) that inhibition of TCTP expression by antisense DNA led to a 'flat rever-

Table 2. TCTP Levels in Human Cancers

Cancer Types	TCTP Levels	Method/Comment	Reference
Colorectal and lung cancer; Oesophageal, hepatocellular, pancreatic cancer	up-regulated not altered	Proteomics studies on tissues and body fluids	[110]
Six Lung SSCs vs. normal tissue; Five Lung adenocarcinomas	usually up-regulated	Western blot of cell lysates	[75]
Serum of lung cancer patients vs. serum of healthy controls	about 2.3-fold up-regulated	Western blot; densitometry	[75]
Breast cancer vs. surrounding tissue	up-regulated	Proteomics study	[111]
Breast cancer	high TCTP associated with aggressive tumours; poor prognosis	Immunohistochemistry	[59]
Tumours of liver, lung, thyroid, larynx, skin, uterus, breast, ovary, prostate, rectum, Tumours of cervix, pancreas, stomach, testis	up-regulated not up-regulated	Western blot of cancer samples vs. normal tissue	[56]
Liver cancer vs. adjacent normal tissue	up-regulated	mRNA levels by RT-PCR analysis	[64]
Hepatocellular carcinoma	up-regulated in most of the advanced tumours	Immunohistochemistry	[24]
Colorectal cancer (20 tumour samples)	2.0-fold up-regulated 1.2-fold up-regulated	mRNA levels by microarray; by real-time PCR	[112]

sion phenotype' of transformed NIH3T3 cells and (3.) that reduction of TCTP levels raised the number of spontaneous revertants dramatically [77]. These results were corroborated by a recent study using a proteomics approach to identify proteins altered in tumour reversion in multiple myeloma cells. This group reported that, among others, STAT3, TCTP, CDC2 and PCNA are down-regulated in the tumour reversion process [78].

• *Mechanisms Through which TCTP Could Promote Cancer*

The most obvious way, by which TCTP is likely to promote cancer, is through its anti-apoptotic activity. In analogy to many other anti-apoptotic proteins, such as Bcl-2, Mcl-1 or Bcl-XL, overexpression of TCTP is part of the armoury of cancer cells to evade apoptosis. For example in LNCaP prostate cancer cells, decreased expression of TCTP was associated with reduced cell viability [79]. Similarly, Lucibello *et al.* [48] demonstrated that cell clones with forced TCTP expression, derived from the breast cancer cell line MDA-MB-231, displayed reduced sensitivity to oxidative stress, whereas cells with down-regulated TCTP showed increased sensitivity.

Another study also underscored the importance of preventing apoptosis for the role of TCTP in cancer. Lee *et al.* [80] established that TCTP (fortilin) interacts with transforming growth factor-beta stimulated clone-22 (TSC-22). Overexpression of TCTP in ovarian carcinoma cells resulted in increased degradation of TSC-22 and the reversal of TSC-22-mediated apoptosis, whereas knockdown of TCTP led to an increase in apoptosis in these cells.

The antagonism between TCTP and the tumour suppressor protein P53 is likely to play a considerable role in TCTP's ability to promote cancer. Substantial evidence for this conclusion has been provided in the recently published paper by Amson *et al.* [59]. These authors found that in about 500 breast cancer patients, a high-TCTP status is associated with aggressive tumours, and predicts a poor prognosis. Also, TCTP knockdown in primary mammary tumour cells from ErbB2 transgenic mice resulted in increased P53 expression and a decreased number of stem cell-like cancer cells.

Ma *et al.* [81] investigated the effect of TCTP knockdown on proliferation, migration, and invasion properties of colon adenocarcinoma cells. They used 2D gel electrophoresis to identify proteins, whose expression levels are altered after TCTP knockdown and found that components of the ubiquitin-proteasome system, proteins involved in the biosynthesis of cytoskeletal proteins and in tumour metastasis were altered upon TCTP removal.

Another recent study, on human hepatocellular carcinoma cells [24], proposed a different mechanism of TCTP-dependent tumorigenesis. These authors studied the mode of action of the chromodomain helicase DNA binding protein1-like (CHD1L), which is amplified and acts a specific oncogene in >50% of human hepatocellular carcinomas (HCC) and found that CHD1L targets the transcription of the TCTP gene tpt1. Overexpression of TCTP was found in about 41% of human HCC samples, and it highly correlated with the advanced tumour stage of HCC patients. Further investigations revealed that the tumorigenicity of TCTP is linked to a role in mitotic regulation, as described earlier. TCTP induces a faster mitotic exit and chromosome miss-segregation, resulting in chromosome instability.

Thus, these two recently published studies describe two important, but quite different mechanisms of TCTP-dependent cancer development. Amson *et al.* [59] emphasise the importance of TCTP-dependent P53 degradation using the example of breast cancer, whereas Chan *et al.* [24], for hepatocellular carcinoma, describe a novel mode of action of TCTP, based on its role in mitotic cell cycle regulation. A common underlying mechanism appears to be the promotion by TCTP of the ubiquitin-proteasome degradation of proteins crucial for cellular homeostasis or cell cycle progression. In this context, the finding by Ma *et al.* [81] is interest-

ing, that after knock-down of TCTP the expression of components of the ubiquitin-proteasome system is altered.

- ***Is TCTP Suitable as an Anti-Cancer Drug Target?***

The multiple roles/mechanisms described so far for TCTP-dependent tumorigenicity provoke the question of whether TCTP is or can be made a suitable drug target in anti-cancer treatment. Whilst, to my knowledge, no report directly addressing this question has been published as yet, there are a couple of papers touching on this important point. In model studies on prostate cancer cells Gnanasekar *et al.* [79] showed that transfection with TCTP siRNA reduced the viability of these cells. Yao *et al.* [82] performed a comparative proteomic analysis to survey changes in protein expression levels after treatment of three colon cancer cell lines with the anti-cancer drug oxaliplatin. TCTP was among the 21 proteins found to be altered in all three cell lines. Its expression was significantly up-regulated within 24 h, but then gradually decreased from 48 to 72 h. Tuynder and colleagues [77] tested a panel of drugs for their ability to suppress TCTP protein levels in U937 cells, and it appeared that this ability correlated well with the potency of these drugs to induce cytotoxicity in these cells. Taken together, these observations would suggest that TCTP might be suitable as a biomarker in testing the drug efficacy in cellular anti-cancer drug testing.

The suitability of TCTP as direct anti-cancer drug target has been reported for three drugs. Since the earlier discovery of *Plasmodium* TCTP as a potential target for the antimalarial drug artemisinin [83], the interaction of TCTP with this drug has been investigated in more detail [84]. Artemisinin is also considered as an anti-cancer drug [85], and Fujita *et al.* [86] published a study on the interaction of human TCTP (fortilin) with dehydroartemisinin (DHA). They demonstrated that DHA binds to human TCTP and decreases cellular TCTP levels through promoting ubiquitination and proteasome-dependent degradation. In their recent article on the antagonism between TCTP and P53, Amson *et al.* [59] report the targeting of TCTP by the pharmacological compounds sertraline and thioridazine, which interfere with the down-regulation of P53 by TCTP.

Taken together, the wide range of observations summarised in this section, provides a solid justification for the term 'tumour protein' in the name of TCTP.

REGULATION OF CELLULAR TCTP LEVELS

1. Cell Physiological Conditions Resulting in Regulation of TCTP Levels

There is a wealth of publications reporting changes of TCTP levels in response to alterations of cell physiological conditions. Many of the groups now working on TCTP discovered this protein through such type of observations. It would be beyond the scope of this article, to comprehensively review all these reports. This section will briefly summarise the main types of extracellular signals that lead to regulation of TCTP levels.

- ***Growth Signals and Nutrients***

As pointed out above, the induction of TCTP synthesis by cell growth signals is well documented (reviewed in [8]). Many of these studies used growth stimulation of serum-starved cells and foetal calf serum as general source of growth factors, whereas only few studies investigated the potential of individual growth factors to induce TCTP synthesis. Vercoutter-Edouart *et al.* [87] demonstrated that in MCF-7 human breast cancer cells, TCTP was among four proteins up-regulated by fibroblast growth factor-2. Recently, we found that TCTP levels are increased about two-fold after insulin treatment in HT29 colon cancer cells (J. Chen & U.A. Bommer, unpublished observation). Schmidt *et al.* [88] demonstrated that TCTP synthesis is induced by phorbol ester and forskolin, both at the transcriptional and posttranscriptional levels of gene expression, and two other papers reported the regulation of TCTP levels through the vitamin D receptor pathway [89, 90].

There is only sparse information about nutrient regulation of TCTP levels. Back in 2000, Bonnet *et al.* reported down-regulation of TCTP (together with ribosomal protein S6) upon ammonium starvation in fission yeast. However, it is likely that this treatment merely reflects a stress condition rather that a genuine nutritional signal. Our recent study on pancreatic beta-cells demonstrated that TCTP levels are subject to glucose regulation in this cell type [43].

- ***Cell Stress Conditions***

The induction of TCTP in response to various stresses was initially reported more than ten years ago, for mammalian cells [44, 91], as well as for worms [92] and for plants [93]. The types of stresses known to date that result in TCTP induction and/or down-regulation include the following groups:

1. ***Heat shock.*** Induction of TCTP synthesis under heat stress conditions was originally noted in parasitic organisms, such as *Trichinella* [94,95] and *Schistosoma* [41]. A recent study investigated the importance of TCTP for stress tolerance of the cabbage, *Brassica oleracea*, and found that silencing of the TCTP gene by RNAi led to reduced vegetative growth rate and decreased tolerance of cold, high temperature and salt stresses by the cabbage plants [96].

2. ***Oxidative stress.*** The first study reporting an oxidation status-dependent regulation of the TCTP gene was published by Rupec *et al.* [91]. These authors set out to clone genes that are induced under hypoxia, as it occurs in solid tumours. They observed a 12-fold up-regulation of a form of TCTP (P23) mRNA, fused to the mitochondrial 16S ribosomal RNA under conditions of hypoxia. They also found that hypoxia induced up-regulation of TCTP mRNA and 16S RNA individually in HeLa cells.

   Yan *et al.* [97] investigated the oxidative stress response in a non-malignant breast epithelial cell line and in a derived malignant cell line. TCTP (fortilin) was found to be significantly up-regulated after hydrogen peroxide treatment in the transformed cells, but not in the parental cells. Gnanasekar and colleagues [47] reported that TCTP from the filarial parasite *Brugia malayi* has anti-oxidant properties. Nagano-Ito *et al.* [46] found that expression of TCTP

cDNA, confers resistance to hydrogen peroxide treatment to mouse NIH-3T3 fibroblasts. Rid and coworkers observed that in human keratinocytes, under mild oxidative stress, TCTP translocates into the nucleus [90].

A very recent study by Lucibello *et al.* [48] showed that in the breast cancer cell line MDA-MB-231 the sensitivity to oxidative stress was strongly enhanced in cells with reduced TCTP levels, and decreased in cells with high TCTP levels. The authors propose TCTP as a 'stress hallmark' in cancer cells. The same paper also investigated the regulation of TCTP under oxidative stress in a range of tumour cell lines. Depending on the severity of the cell stress, the authors found either up- or down-regulation of TCTP levels: In mild oxidative stress, TCTP was up-regulated in cells that survived the treatment. In contrast, severe oxidative stress caused down-regulation of TCTP followed by cell death. Generalising from these observations it would seem that under mild stress conditions, TCTP is induced as part of the cell's defence system, whereas under severe stress, pro-apoptotic mechanisms prevail, which include the down-regulation of anti-apoptotic proteins, such as TCTP.

3. ***Ca^{++}-stress.*** Regulation of TCTP levels in Ca^{++}-stress conditions has initially been studied by Xu *et al.* [44] in COS-7 cells. They observed an increase in TCTP levels under these conditions, regulated at both transcriptional and post-transcriptional levels. In contrast, in our later study on mouse embryo fibroblasts we found that TCTP levels are down-regulated under calcium ionophore or thapsigargin treatment [42]. Interestingly, in some cases, we also saw a moderate initial up-regulation of TCTP levels, followed by a more substantial down-regulation upon prolonged treatment. The conclusion drawn in the previous paragraph from the paper by Lucibello *et al.* [48] would be consistent with this latter observation, and it could provide an explanation to reconcile the two apparently contradicting results for TCTP regulation in Ca^{++}-stress [42,44]. Decrease of TCTP levels were also observed in pancreatic beta-cells upon stress induced by treatment with thapsigargin or saturated fatty acids, i.e. palmitate [43].

4. ***Stress induced by heavy metals.*** The most dramatic alterations of TCTP levels have been observed under exposure of cells or organisms to heavy metals. The first paper reporting such an example investigated the gene expression pattern of earthworms (*Lumbricus rubellus*) exposed to heavy metal-infested soils from the mining industry. It reported an at least four-fold increase in TCTP (mRNA) levels in a earthworm population from a Pb/Zn/Cd-polluted mine and an even 335-fold increase in earthworms from a Cu-polluted mine [92]. Schmidt *et al.* [88] working with two mammalian cell lines, found that cobalt and nickel induced TCTP expression about two- to three-fold at the mRNA and protein levels, whereas copper resulted in a five-fold induction of TCTP mRNA or protein levels. TCTP was also identified as one of the proteins specifically expressed in plants that are tolerant to aluminium-stress [93].

5. ***Stress induced by toxins or drugs.*** Oikawa *et al.* [98] studied the effect of tetrachlorodibenzo-p-dioxin (TCDD, or 'dioxin') on the mRNA expression patterns of mouse embryonic stem cells. The mRNA of TCTP (HRF) was found to be up-regulated by TCDD, which also induced the synthesis and secretion of TCTP. Another group of toxic and carcinogenic pollutants are the polycyclic aromatic hydrocarbons (PAHs). Zheng *et al.* [99] investigated the effect of PAHs in soil on the expression of TCTP in the earthworm *Eisenia fetida* and found that it was up-regulated by benzopyrene, but down-regulated by phenanthrene. The effect of anti-cancer drugs on TCTP levels was discussed earlier.

2. Mechanisms Involved in Regulation of Cellular TCTP Levels

Whilst TCTP was originally discovered as a translationally regulated protein, it is now overwhelmingly clear that regulation of cellular TCTP levels may occur at several levels of gene expression and through a range of mechanisms. This is not surprising, given the variety of stresses or stimulatory signals that have been described to regulate TCTP levels, and considering the velocity and extent of alteration of cellular TCTP levels. Moreover, this is well in line with observations published for other anti-apoptotic or stress proteins, which are also regulated at multiple levels. Here, I will focus on the major mechanisms known to-date to be involved in TCTP regulation.

- ***Gene Regulation at the Transcriptional Level***

The most detailed studies on gene structure and transcriptional regulation of TCTP synthesis were published by the Thiele laboratory in Berlin. Initially, they described the gene structure and upstream promoter sequences of the TCTP gene from rabbit and humans and established that these regions contain several promoter elements that are well-conserved in mammals. They also showed that, as a whole, this upstream region exhibits a strong promoter activity [100]. In a second report, the group characterised various features of TCTP mRNAs. Typically, cells generate two forms of this mRNA that differ in the length of their 3'-untranslated regions. These were found to be transcribed in all rabbit and human tissues investigated, but at a considerable range of overall quantity and ratio of expression [73]. This paper also characterises four of the numerous processed, intronless pseudogenes of TCTP in the rabbit genome. This earlier work is summarised in our previous review article [8]. A similar paper, characterising the genomic structure and several pseudogenes of the mouse TCTP gene, was published at the same time by the Telerman group [101].

Additional evidence for transcriptional regulation of TCTP levels under specific physiological conditions came from other publications, such as Stuerzenbaum *et al.* [92], who found extreme differences in TCTP mRNA levels in earthworms exposed to soils polluted with specific heavy metals, as discussed above. Xu *et al.* [44] reported both transcriptional and translational regulation of TCTP levels under

Ca^{++}-stress situations in COS-7 cells. Bonnet *et al.* [102] studied genes that are specifically regulated under ammonium starvation in the fission yeast *S. pombe*, which leads to growth arrest and cell cycle exit. They found that the genes for ribosomal protein rpS6 and TCTP are repressed under this condition, and that their expression is co-ordinately regulated in many different growth conditions. Under ammonium starvation, transcriptional inhibition of these genes was regulated either by the PKA pathway and/or by the Wis1/MAP kinase pathway [102]. Oikawa *et al.* [98] showed that the dioxin-stimulated expression of TCTP (HRF) mRNA occurs via an aryl hydrocarbon receptor (AhR)-dependent pathway.

These were the first reports on specific signalling pathways/transcription factors that are involved in transcriptional activation of the TCTP gene. A further study of the promoter region of the human TCTP gene, again by the Thiele group, revealed that transcriptional regulation of TCTP occurs by PKA signalling, via activation of members of the CREB family of transcription factors [103], which is consistent with the conclusions obtained from fission yeast mutants mentioned above [102]. Other transcription factors recently shown to directly regulate the transcription of the tpt-1 (TCTP) gene include CHD1L [24] and the tumour suppressor protein p53 [59].

Another paper from the Thiele laboratory investigated both transcriptional and post-transcriptional regulation of the tpt-1 (TCTP) gene in mammalian cell lines under a variety of conditions known to result in alterations of TCTP levels [88]. Phorbol ester, forskolin, dioxin, cobalt, nickel and, to the highest extent, copper induced TCTP expression at both the mRNA and protein levels. The strong copper-dependent transcriptional activation was transmitted by a metal-response element residing in the tpt-1 promoter. In contrast, the more moderate effects by cobalt and nickel where mediated by mRNA stabilisation, rather than by transcriptional activation [88].

- ***Translational Regulation of TCTP Levels***

Early evidence for translational regulation of TCTP emerged from the following observations: 1. The serum-induced increase in the rate of TCTP (P23 or Q23) synthesis clearly preceded that of transcriptionally regulated proteins, and it was not inhibited by actinomycin D [1, 3]. 2. The mRNA of TCTP (P21) was found abundantly in untranslated cytoplasmic mRNP particles [2], which are presumed to be a cytoplasmic 'reserve pool' of untranslated mRNAs.

At that time, knowledge about translational control mechanisms was just beginning to emerge. In the early nineties, the importance of the cap-binding initiation factor eIF4E for the efficient translation of otherwise poorly translated mRNAs was just being appreciated. Such mRNAs typically code for proteins, which are involved in the cell cycle and cancer (reviewed in [104]). Since TCTP (P23) was described as growth-induced translationally controlled protein, we investigated whether it might be one of the mRNAs specifically regulated by eIF4E. We made use of eIF4E overexpressing fibroblasts to demonstrate that the rate of synthesis of TCTP is indeed correlated with eIF4E levels/activity in the cell [105].

Since this publication, important additional elements of the signalling pathways and mechanisms that regulate protein synthesis have been elucidated. It has become clear that the mammalian target of rapamycin complex 1, mTORC1, is central to the complex regulatory network for protein synthesis and that eIF4E is an important element of this network, downstream of mTORC1. The eIF4E binding proteins (eIF4E-BPs), which normally inhibit eIF4E, are inactivated through phosphorylation by mTORC1 (reviewed in [65]).

There are two key features of the TCTP mRNA that make it a prime candidate for an mRNA specifically regulated by mTOR (and eIF4E): 1. As mentioned above, TCTP mRNA has a 5-terminal oligopyrimidine tract (5'-TOP), the signature typical for mRNAs coding for proteins that are part of the translational machinery and are known to be regulated by mTORC1. 2. We have previously shown that TCTP mRNA is a highly structured RNA molecule [60], and translation of such mRNAs is particularly dependent on the activity of eIF4E.

For these reasons, we are currently reinvestigating the mechanisms involved in growth-factor-dependent translational up-regulation of TCTP synthesis; in particular we address the question of the involvement of the mTORC1 pathway. We have now obtained results demonstrating that in HeLa cells, serum-induced synthesis of TCTP is partially inhibited by rapamycin, and completely inhibited by inhibitors of mTOR kinase activity and of the protein kinase Akt, which is upstream of mTORC1. In addition, eIF4E overexpression drives TCTP mRNA from the subpolysomal fraction into actively translating polysomes (U.A. Bommer, V. Iadevaia and C.G. Proud, unpublished results). These data indicate that TCTP mRNA behaves like a genuine 5'-TOP mRNA that is regulated by mTORC1.

Another key regulatory mechanism of translation, which leads to cell stress-dependent down-regulation of protein synthesis, involves the phosphorylation of the α-subunit eukaryotic initiation factor eIF2. There are four distinct eIF2α kinases, which are each activated under specific cell stress conditions. We have studied the effect of the double-stranded RNA-dependent protein kinase PKR on TCTP mRNA translation [60] and have shown that the highly structured TCTP mRNA is able to bind to and activate PKR, resulting in its own translational inhibition. We have also demonstrated that PKR is indeed necessary for the down-regulation of TCTP synthesis under specific stress conditions, such as serum starvation [60] or Ca^{++}-stress conditions [42].

The involvement of these two key regulatory mechanisms in the regulation of intracellular TCTP levels is in line with corresponding observations for other anti-apoptotic proteins. For example, it is well established that the synthesis of proteins like Bcl-XL, Mcl-1 and survivin are regulated through the mTORC1 pathway and require eIF4E activity for their translation [106]. Synthesis of Mcl-1 is also down-regulated by PKR [107].

- ***Other Post-Transcriptional Regulatory Mechanisms***

Schmidt *et al.* [88] investigated the mechanisms involved in the regulation of TCTP by heavy metals, and found that

the moderate inductive effects by cobalt and nickel are mediated by mRNA stabilisation, whereas the strong inductive effect by copper was mediated both at transcriptional and posttranscriptional levels. They speculated that the AUUUA elements present in the 3'-untranslated region of TCTP mRNAs might be involved in the regulation of TCTP mRNA stability. However, these AUUUA elements do not completely match the features of canonical AU-rich elements (AREs) known to be involved in the destabilisation of cytokine mRNAs, and nobody has this far directly investigated their importance in TCTP mRNA. Generally, TCTP mRNA has been found to be fairly stable and abundant in mammalian cells, although the abundance may vary considerably, depending on the tissue type [73]. Thus, mRNA stability regulation as potentially effective regulatory mechanism for TCTP protein levels should be looked at in the context of the specific tissue and regulatory condition.

There are few reports describing the regulation of TCTP levels through protein degradation. Kubiak and colleagues [18] set out to characterise proteins that are specifically degraded in the first embryonic mitosis in *Xenopus laevis*. They identified TCTP as one of the proteins that are ubiquitinylated and potentially degraded via the proteasome in this phase of the cell cycle. Zhang *et al.* [52] described the interaction of TCTP (fortilin) with the anti-apoptotic protein Mcl-1 and demonstrated in Mcl-1 knockdown experiments that TCTP is stabilised by Mcl-1. The same group reported later that dehydroartemisinin (DHA), an important anti-malarial drug, which was previously shown to bind to *Plasmodium falciparum* TCTP, binds to and destabilises human TCTP in a proteasome-dependent manner [86]. This is interesting, since DHA is also considered an anti-cancer agent [85, 108]. Although the reports on regulated protein degradation of TCTP are limited and anecdotal so far, it appears that this type of regulation may occur in a distinct cell cycle phase and under pro-apoptotic cell stress conditions.

CONCLUDING REMARKS

After a slow beginning, research on the translationally controlled protein TCTP has accelerated considerably over the past 30 years, with a large number of groups having contributed many different facets to the 'TCTP story'. Functional importance has been attributed to this protein in a range of biological contexts, on both the molecular and the cellular levels. Since TCTP plays a major role in cell growth, division and reactions to stress situations, its cellular levels are subject to a considerable degree of regulation. It is therefore not surprising that TCTP regulation occurs at several levels of gene expression and engages a range of mechanisms. TCTP's importance for cellular homeostasis is further reflected at the organismic level, as obvious from its involvement in diseases processes, such as cancer or allergy. It will be important to better understand the molecular interactions and cellular roles of TCTP, and how they underpin such disease processes.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

Work in my laboratory was supported by grants from The Wellcome Trust, by a Short-Term Fellowship from the Human Frontier Science Program (Strasbourg), and by small grants from the Cancer Prevention Research Trust (London, UK), from the Graduate School of Medicine and the Illawarra Health and Medical Research Institute, University of Wollongong. I thank Professors Chris Proud (Southampton) and Thomas Preiss (Canberra) for helpful comments on parts of the manuscript.

NOTE ADDED IN PROOF

While this article was in press, a new paper was published, documenting a novel function for TCTP: Zhang J, de Toledo SM, Pandey BN, Guo G, Pain D, Hong Lie, and Azzam EI. Role of the translationally controlled tumor protein in DNA damage sensing and repair. Proc. Natl. Acad. Sci. USA 2012 (www.pnas.org/cgi/doi/10.1073/pnas.11063-00109 <http://www.pnas.org/cgi/doi/10.1073/pnas.1106300-109>).

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Received: January 25, 2012 Revised: February 10, 2012 Accepted: February 17, 2012