Expression of translationally controlled tumour protein is regulated by calcium at both the transcriptional and post-transcriptional level

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We have investigated how the programme of protein synthesis is altered in response to a loss of calcium homoeostasis in Cos-7 cells using a differential proteome mapping approach. Exposure of the cells to the calcium ionophore A23187 or thapsigargin, or alternatively, expression of a viral glycoprotein reported to deplete intracellular calcium stores, resulted in the up-regulated expression of a characteristic set of proteins. One of these is the translationally controlled tumour protein (TCTP), a cytoplasmic protein whose expression has not previously been linked to calcium perturbation. Quantitative Northern blot assay demonstrated that steady-state mRNA abundance of TCTP was also increased under these conditions. Clamping the cytosolic calcium concentration by the introduction of bis-(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid (BAPTA) into cells did not affect the increase in steady-state levels of TCTP mRNA observed in response to ionophore. Therefore depletion of endoplasmic reticulum (ER) calcium, but not elevation of the cytosolic calcium concentration, was responsible for increased transcription of the TCTP gene. However, the presence of BAPTA significantly attenuated the ionophore-mediated increase in levels of the protein. Moreover, the level of TCTP in ionophore-treated cells increased in advance of a detectable increase in the corresponding mRNA abundance. These results indicate that expression of TCTP is regulated at two distinct levels in response to the concentration of calcium in different cellular compartments. Whereas depletion of the ER store causes an increase in TCTP mRNA abundance, increased cytosolic calcium concentrations regulate gene expression at the post-transcriptional level.

Key words: endoplasmic reticulum stress, gene expression, TCTP, transcription.

INTRODUCTION

Calcium ions (Ca^{2+}) play a key role in the regulation of cellular metabolism and gene expression. The concentration of Ca^{2+} in the cytosol of eukaryotic cells (typically 10–100 nM) is maintained in homoeostatic balance with millimolar Ca^{2+} levels in both the extracellular environment and the lumen of the endoplasmic reticulum (ER), the major dynamic Ca^{2+} storage compartment of non-muscle cells [1]. The rapidly exchanging Ca^{2+} store in this organelle is essential for a variety of cellular functions, including correct folding and post-translational modification of newly synthesized secretory and membrane proteins, translation of newly transcribed mRNA and in maintaining the structural integrity of the ER [1,2]. The intracellular calcium store participates in the regulation of the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) through rapid Ca^{2+} release and by controlling store-operated Ca^{2+} influx from outside the cell [3–5]. At the same time, the high Ca^{2+} concentration within the ER is sustained by the action of pumps that drive Ca^{2+} uptake, [the so-called SERCAs (sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPases)], and a group of high-capacity, low-affinity Ca^{2+}-binding proteins that buffer Ca^{2+} in the ER lumen [1].

Disruption of Ca^{2+} homoeostasis impairs the normal functioning of the cell. A sustained elevation of cytosolic Ca^{2+} can cause the activation of degradative enzymes and can lead to both apoptotic and necrotic cell death [6]. Similarly, depletion of Ca^{2+} from the ER can impair the functional status of this organelle and impose a Ca^{2+} stress on the cell. In response to store depletion, a number of signal-transduction pathways may be activated, resulting in the transactivation of a subset of genes through common calcium-responsive promoter elements [7,8]. A classical hallmark of ER perturbation, including Ca^{2+} stress, is the induction of a group of ER-resident Ca^{2+}-binding proteins. These include immunoglobulin heavy-chain binding protein/glucose-regulated protein (BiP/GRP78) and calreticulin, which account for $\sim 30\,\%$ and $\sim 50\,\%$ of the total ER Ca^{2+}-buffering capacity respectively [9,10]. Changes in the programme of gene expression triggered by depletion of Ca^{2+} from the ER store may therefore be viewed in part as a protective response designed to restore Ca^{2+} levels in this compartment. Many of these ER-resident binding proteins also possess molecular chaperone activity and their presence is essential for correct folding and assembly of nascent polypeptides within the ER lumen [8]. The presence of unfolded proteins in the ER may act as a primary signal for the induction of a stress-response pathway which, in yeast, is referred to as the unfolded protein response (UPR) [7].

The response of mammalian cells to ER stress is complex, with multiple signals able to trigger a range of overlapping pathways. For example, the pleotrophic transcription factor NF-κB is activated by a range of conditions that perturb ER function (the so-called ER-overload response), reflecting the existence of an ER–nuclear signalling pathway distinct from the UPR [7,11]. Similarly, CHOP [C/EBP (CCAAT-enhancer-binding protein) homology protein], a transcription factor involved in pro-

Abbreviations used: BAPTA, bis-(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid; BiP, immunoglobulin heavy-chain binding protein; $[Ca^{2+}]_i$, cytosolic free Ca^{2+} concentration; 2-DE, two-dimensional gel electrophoresis; DTT, dithiothreitol; ER, endoplasmic reticulum; GRP, glucose-regulated protein; TCTP, translationally controlled tumour protein; UPR, unfolded protein response.

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grammed cell death, is activated following Ca^{2+} depletion or other inducers of ER stress [12–14]. Our understanding of the changes in cellular gene expression and the associated phenotypic changes that accompany depletion of ER Ca^{2+} is far from complete. The identification of novel genes differentially expressed under such circumstances and a better understanding of their regulation will assist in clarifying details of this response.

Calcium stress is a feature of numerous pathological conditions, including viral infection and in some cases, expression of individual viral proteins [15–18]. For example, rotavirus infection causes a profound elevation of cytoplasmic Ca^{2+} levels which are linked to the virus cytopathic effect [16,19]. Recombinant expression of NSP4, a rotavirus non-structural glycoprotein, has also been demonstrated to alter $[Ca^{2+}]_i$ by causing depletion of the ER stores [20,21]. Both rotavirus infection and expression of NSP4, using a recombinant vaccinia virus vector, cause an increase in the expression of several ER-resident chaperones associated with activation of the UPR [22]. Here we report that transfection of Cos-7 cells with a gene encoding NSP4 increases expression of a protein not previously recognized as belonging to this group of Ca^{2+} stress proteins. We have also identified a novel facet of Ca^{2+}-regulated gene expression in which the concentration of Ca^{2+} in both the ER and cytosol can regulate the expression of a single gene at the transcriptional and post-transcriptional levels respectively.

MATERIALS AND METHODS

Reagents

Calcium ionophore A23187 and tunicamycin were obtained from Sigma. Thapsigargin and BAPTA-AM (bis-(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid acetoxymethyl ester) were from Molecular Probes. All radioactive isotopes ($^{45}CaCl_2$, ^{35}S Trans-label and ^{32}P-dCTP) were obtained from ICN.

Plasmid construction and transient transfection of Cos-7 cells

A cDNA fragment encoding the rotavirus NSP4 gene from strain SA11 [23], was obtained by PCR amplification of the gene cloned in pBluescript using the following primer pair: 5′GCAAGCTTAACCATGGAAAAACTTACCGACCCCT3′ (sense) and 5′-TGGATCCTTACATTGCTGCAGTCACTTCTCT3′ (antisense). The amplified gene lacks the internal *Hin*dIII site which is relocated upstream of the ATG by the sense primer. The PCR product was cut with *Hin*dIII and *Bam*HI and cloned in pCDNA3.1 (Invitrogen) to yield pCNSP4. For expression of NSP4, Cos-7 cells were transfected with pCNSP4 or the parental plasmid using Lipofectamine Plus (Life Technologies) according to the manufacturer's instructions.

Immunoprecipitation and immunofluorescence

Transfected cells were labelled with ^{35}S Trans-label for 3 h, commencing 45 h after transfection. NSP4 was precipitated from ^{35}S-labelled cell lysates using an anti-NSP4 polyclonal antibody as described previously [22]. For immunofluorescence microscopy, the transfected cells were fixed in 3% paraformaldehyde 48 h after transfection, permeabilized with 0.2% (v/v) Triton X-100 and stained using anti-NSP4 polyclonal antibody and rhodamine-conjugated anti-rabbit IgG (Amersham). To confirm ER localization of NSP4, the staining pattern was compared with that of the endogenous ER marker protein endoplasmin.

Two-dimensional gel electrophoresis (2-DE) and proteome analysis

Protocols for preparation of cell lysates, protein quantification and 2-DE were as previously described [22], with the following alterations. To improve sample solubility and avoid protein precipitation, 2 M thiourea was included in the lysis buffer and samples were loaded by direct absorption into the first-dimensional strip during the rehydration step. Immobiline Drystrip 4–7 L gels were used throughout. After electrophoresis, proteins in the gels were visualized by autoradiography, phosphorimaging (Fujifilm FLA 2000) or staining with Coomassie Brilliant Blue R250. Autoradiography films were scanned and digitized using a Sharp JX 325 scanner. Protein spots were detected, quantified and analysed using Imagemaster™ software (Pharmacia). Radioactivity associated with selected protein spots was also quantified using a phosphorimager (BAS 2000; Fujifilm). To identify proteins of interest, the relevant spots were excised from Coomassie Brilliant Blue-stained gels. Gel pieces were pooled and subjected to in-gel trypsinization followed by reverse-phase HPLC, as previously described. Only well-resolved peaks from each sample were applied for N-terminal amino acid sequencing.

Northern blot analysis

A translationally controlled tumour protein (TCTP) cDNA probe was generated by reverse-transcriptase PCR (Ready-to-go, Pharmacia) using primers complementary to the human TCTP gene sequence [24] in a reaction containing 5 μg of HeLa mRNA. Extraction of total cytoplasmic RNA and hybridization were performed according to a previously described protocol [22]. All membranes were later stripped and rehybridized with an actin probe to calibrate mRNA.

^{45}Ca overlay assay

Cell lysates from A23187-treated cells were resolved by 2-DE and the proteins were transferred to nitrocellulose membrane using a Multiphor RII semi-dry blotter apparatus (Pharmacia). The membranes were rinsed briefly with blotting buffer to remove residual acrylamide, washed three times in buffer (60 mM KCl/5 mM $MgCl_2$/10 mM imidazole/HCl, pH 6.8) for 20 min each and subsequently incubated in the same buffer containing 2 μCi/ml $^{45}CaCl_2$ for 20 min. The radioactive solution was removed and the membranes were washed in aqueous ethanol for 5 min, dried completely and exposed to Kodak X-ray film. After autoradiography the membranes were stained with Amido Black.

RESULTS

Transient expression of rotavirus NSP4 in Cos-7 cells

The initial objective of this study was the identification of cellular polypeptides differentially expressed in response to NSP4 expression. NSP4 has been identified as an ER-localized membrane glycoprotein [25]. When delivered by a recombinant vaccinia virus, NSP4 activates the transcription of several genes encoding ER-resident stress proteins in MA104 cells [22]. Therefore transfection of the cloned NSP4 cDNA was used to drive the expression of NSP4 in Cos-7 cells and changes in the programme of cellular protein synthesis were monitored using a proteome mapping approach based on the resolution of cellular proteins by 2-DE. Immunoprecipitation of ^{35}S-labelled protein with anti-NSP4 antibodies revealed the existence of a major 28 kDa species corresponding to glycosylated NSP4 and a minor amount of a 20 kDa unglycosylated form (Figure 1A). Immuno-

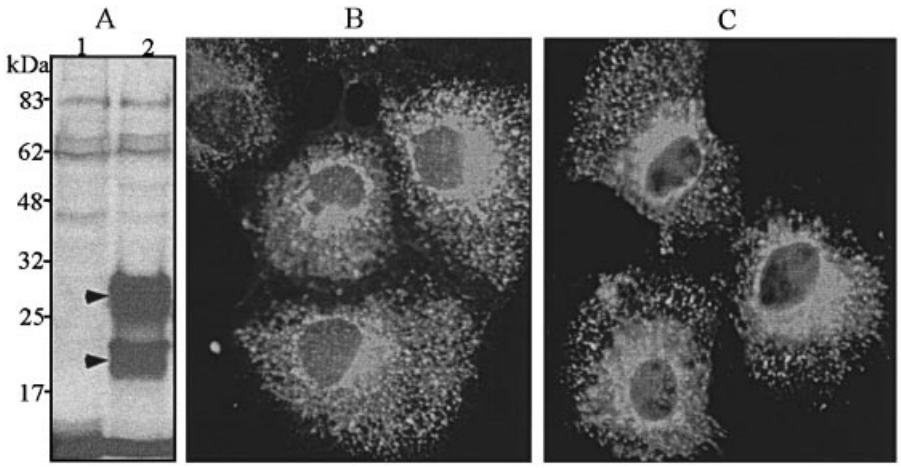


Figure 1 Expression of rotavirus NSP4 in Cos-7 cells

(**A**) Autoradiography of immunoprecipitated ^{35}S-labelled NSP4. Cells were transfected with pCDNA3.1 (lane 1) or pCNSP4 (lane 2). Proteins were precipitated by anti-NSP4 polyclonal antibody and resolved by SDS/PAGE. Arrows indicate the position of glycosylated (upper) and unglycosylated (lower) NSP4. (**B**) Localization of NSP4 transiently expressed in COS-7 cells using immunofluorescence microscopy. (**C**) Immunofluorescent localization of the ER-resident protein endoplasmin.

fluorescence microscopy showed a transfection efficiency of $\sim 80\,\%$. The cellular distribution of NSP4 (Figure 1B) was found to be similar to that of endoplasmin, an endogenous ER-resident protein [8] (Figure 1C).

Expression of NSP4 up-regulates the synthesis of several cellular proteins

^{35}S-labelled proteins from NSP4-transfected and control cells were resolved by 2-DE and visualized by autoradiography. Proteins whose expression increased at least 3-fold following NSP4 expression were identified using Imagemaster software (Pharmacia) and selected for identification. This analysis revealed that seven acidic proteins located within a similar region of the gel were reproducibly up-regulated (Figure 2). Further quantitative analysis revealed that the amount of radioactivity associated with each of these proteins increased 3–10-fold. Six of these up-regulated proteins were unequivocally identified on the basis of amino acid sequence data obtained from tryptic peptides eluted from the relevant gel spot (Table 1). Five of these [170 kDa GRP, endoplasmin, BiP, calreticulin, endoplasmic reticulum protein 5 (ERP5)], correspond to proteins whose status as ER-resident molecular chaperones has been described [8,26–28]. A further protein (spot 7, Figure 2), induced in response to NSP4

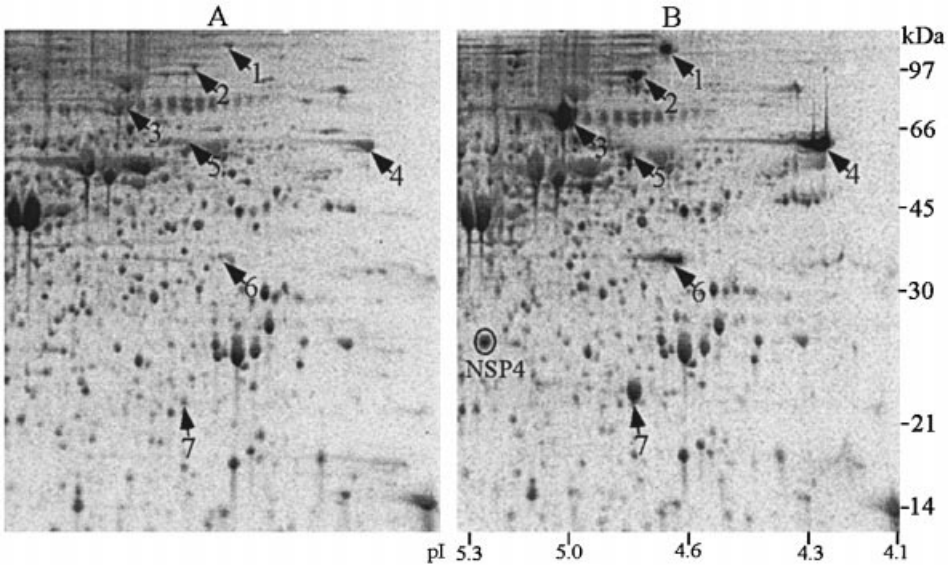


Figure 2 Expression of NSP4 up-regulates synthesis of several cellular proteins in Cos-7 cells

Cells were transfected with pCDNA3.1 (**A**) or pCNSP4 (**B**) and an equal amount of ^{35}S-labelled cell lysates were analysed as described in the Materials and methods section. The positions of up-regulated cellular proteins are denoted by numbered arrows. A typical result is shown from three independent experiments.

Table 1 Identification of up-regulated proteins in pCNSP4-transfected Cos-7 cells

Spot no.*	Amino acid sequence	Protein†	Ref.
1	LGNTISSL	GRP170	[26]
2	SGTSEFLN	Endoplasmin (GRP94)	[41]
3	IEWLESHQ	BiP (GRP78)	[43]
4	IKDPDASK	Calreticulin	[42]
5	HQSLGG	ERP5	[27]
7‡	TEGNIDDS	TCTP	[34]
7‡	EIADGL	TCTP	[34]

* Numbers refer to protein spots identified in Figure 2.

† Amino acid sequences were matched to entries in the SwissProt-PIR database (http://www.expasy.hcuge.ch).

‡ Two peptides of spot 7 were sequenced to confirm the identity.

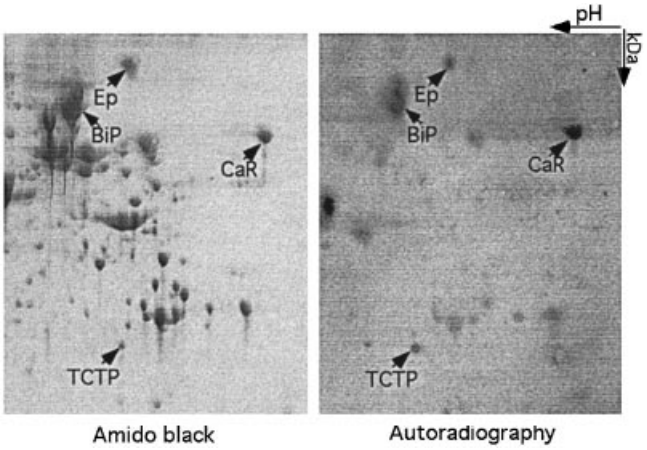


Figure 3 Detection of calcium-binding proteins in Cos-7 cells

Proteins from A23187-treated cells were resolved by 2-DE and transferred to nitrocellulose membrane. Ca^{2+}-binding proteins were identified as described in the Materials and methods section. The positions of BiP, endoplasmin (Ep), calreticulin (CaR) and TCTP are indicated.

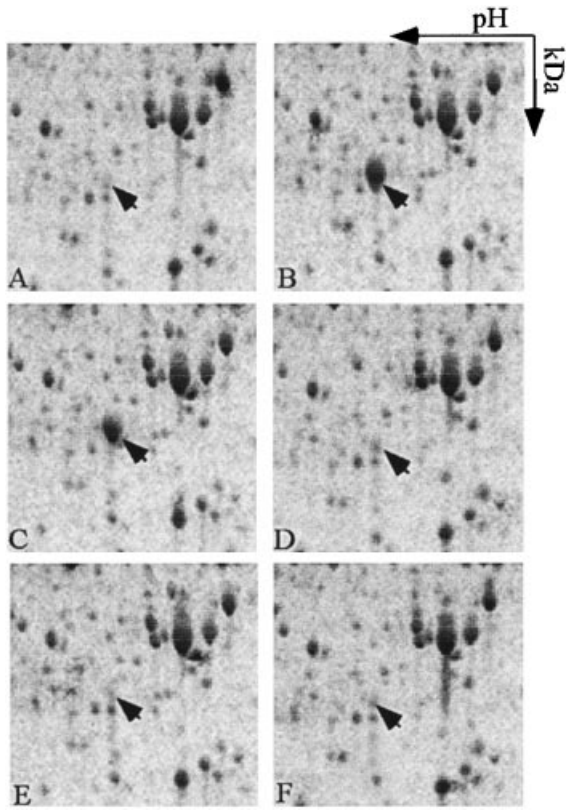


Figure 4 Effects of various stress conditions on TCTP synthesis

Control cells (**A**), and cells incubated with 7 μM A23187 (**B**), 200 nM thapsigargin (**C**), 500 μM DTT (**D**), 2 μg/ml tunicamycin (**E**) for a total of 8 h or heat stressed at 42 °C for 24 h (**F**). All cells were labelled with ^{35}S for 2 h and proteins were resolved by 2-DE. The position of TCTP in each gel is indicated by an arrow. The single result shown is representative of results obtained in three independent experiments.

expression, was identified as TCTP. TCTP is a cytoplasmic protein and may associate with cytoskeletal structures [29,30].

TCTP is a Ca^{2+}-binding protein

The ER-resident proteins up-regulated in Cos-7 cells following NSP4 expression share the ability to bind Ca^{2+}. Although the primary sequence of TCTP does not reveal any recognizable Ca^{2+}-binding motif, previous studies have demonstrated a Ca^{2+}-binding property for a recombinant form of human TCTP expressed in *Escherichia coli* and in a close homologue of TCTP from *Trypanosoma brucei* [30]. We utilized a $^{45}Ca^{2+}$ overlay assay to determine whether TCTP of mammalian origin shared this capability. The result showed that, although many cellular proteins were resolved by 2-DE, $^{45}Ca^{2+}$ was selectively retained by only a small set of proteins, including TCTP and several recognized ER-resident Ca^{2+}-binding proteins (Figure 3). Therefore, we conclude that TCTP may represent a novel type of Ca^{2+}-binding protein in mammalian cells.

Effect of several ER stress conditions on expression of TCTP

The up-regulated expression of several ER-resident proteins is a characteristic feature of the cellular response to ER stress induced by a range of perturbants of organelle function. We therefore tested whether TCTP was induced specifically by NSP4 or whether a range of different stress-inducing conditions could mimic this effect. Cos-7 cells were individually exposed to the following for a period of 8 h: 7 μM calcium ionophore A23187, 200 nM ER Ca^{2+}-ATPase inhibitor thapsigargin, 2 μg/ml tunicamycin or 500 μM dithiothreitol (DTT). Alternatively, cells were heat-shocked at 42 °C for 20 h. After treatment, the cells were labelled for 2 h and proteins were analysed by 2-DE. TCTP expression was increased in response to A23187 (8.4-fold) or thapsigargin (7.3-fold) but was not affected by DTT, tunicamycin or heat treatment (Figure 4). Both A23187 and thapsigargin deplete the ER of stored Ca^{2+}. On the other hand, tunicamycin, an inhibitor of N-linked glycosylation, and DTT, a reducing agent, inhibit correct folding and assembly of glycoproteins within the ER and thus activate the UPR. We conclude that up-regulation of TCTP expression is linked specifically to altered cellular Ca^{2+} homoeostasis and is not a feature of the more general ER stress response caused by the accumulation of unfolded proteins.

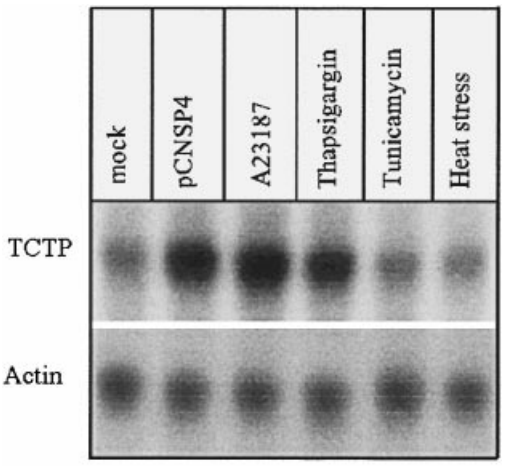


Figure 5 Induction of TCTP transcription by Ca^{2+} perturbation reagents

Cytoplasmic total RNA was extracted from Cos-7 cells either transfected with pCNSP4, treated with various reagents, or subjected to heat stress. RNA was resolved by electrophoresis, transferred to Nylon membrane and hybridized with ^{32}P-labelled TCTP and actin probes.

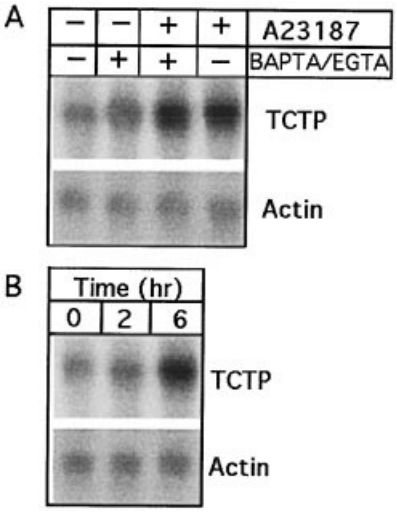


Figure 6 Increase in steady-state levels of TCTP mRNA following depletion of Ca^{2+} from the ER

(**A**) Effect of buffering cytosolic Ca^{2+} on A23187-stimulated transcription of TCTP gene. Exposure to A23187 (7 μM) was for 8 h. (**B**) Change in TCTP mRNA levels over time in A23187-treated Cos-7 cells. Note cells loaded with the cytosolic Ca^{2+} chelator BAPTA were incubated in Ca^{2+}-free growth medium that contained 5 mM EGTA for the duration of the experiment.

Depletion of stored Ca^{2+} induces transcription of the TCTP gene

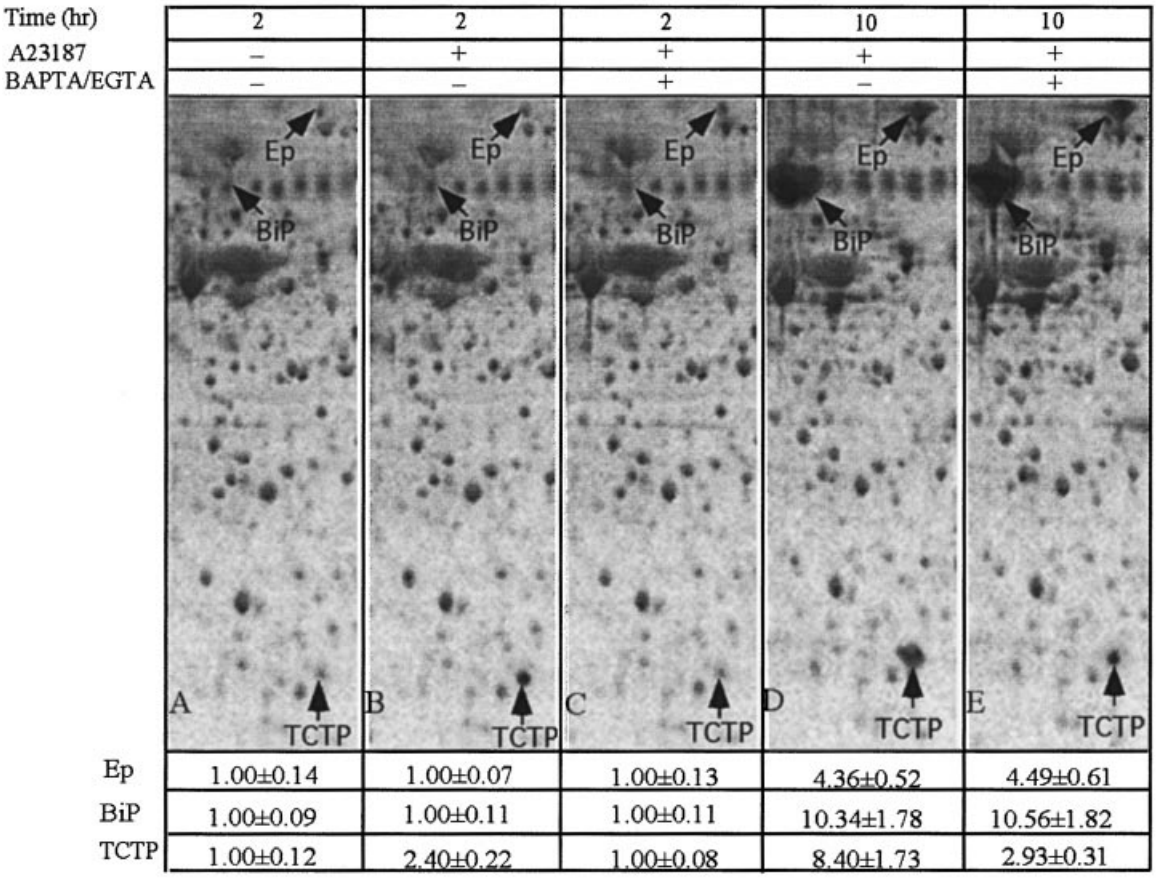
To determine the level at which expression of TCTP is regulated by Ca^{2+} stress, we analysed mRNA abundance by quantitative Northern blot analysis (Figure 5). Steady-state levels of TCTP mRNA increased 3.5-fold in response to NSP4 expression. TCTP mRNA abundance was also increased after treatment of cells with A23187 (3.1-fold) and thapsigargin (2.8-fold), but was not altered in response to DTT, tunicamycin or heat treatment. Next we sought to establish whether increased TCTP mRNA abundance arose specifically from depletion of Ca^{2+} from the ER lumen or was due to a resultant elevation of the cytosolic Ca^{2+} concentration. Cells were incubated in Ca^{2+}-free medium and loaded with the cytosolic Ca^{2+} chelator BAPTA, before the addition of A23187, to buffer the ionophore-induced increase in cytosolic Ca^{2+} levels. Under these conditions the increase in TCTP mRNA abundance in response to A23187 was comparable with that observed in control cells free of BAPTA (3.3- and 3.1-fold respectively) (Figure 6A). Furthermore, time course studies showed that the abundance of TCTP mRNA did not increase within 2 h after A23187 treatment (Figure 6B). Collectively, these results demonstrate that the increased synthesis of TCTP mRNA results specifically from depletion of ER Ca^{2+} and not from an increase in $[Ca^{2+}]_i$.

Elevation of $[Ca^{2+}]_i$ increases TCTP expression at a post-transcriptional step

Comparison of the steady-state levels of TCTP mRNA with the corresponding amount of protein synthesized under identical conditions suggested that increased transcription of the TCTP gene may not fully account for the increase in the abundance of the protein after exposure to cells with ionophore. For example, after an 8 h incubation of the cells with A23187, the level of TCTP was increased 8.4-fold compared with a 3.1-fold increase in the abundance of the corresponding mRNA. Previously, regulation of TCTP expression has been reported to occur at the translational level [31,32]. We therefore investigated whether perturbation of intracellular Ca^{2+} homoeostasis could also influence expression of TCTP at the post-transcriptional stage. To address this question, the quantity of ^{35}S-labelled TCTP synthesized during a given period following exposure to ionophore was measured. The increase in levels of TCTP was compared with those of endoplasmin and BiP in the same cells. The relative abundance of TCTP in cells exposed to A23187 for a 2 h period increased 2.4-fold over basal levels (Figure 7, compare A and B). This increase occurred in advance of a detectable rise in TCTP mRNA abundance, suggesting that Ca^{2+} might regulate expression at a post-transcriptional level. We observed that within a 2 h period immediately after addition of ionophore to the cells, the total amount of protein synthesized was approx. half that in ionophore-free control cells (results not shown). This result was not unexpected, since the transient inhibition of translation due to activation of protein kinase R and phosphorylation of the elongation initiation factor eIF2α in response to calcium ionophore has been described [2]. In a second experiment, we examined the relative expression level of the same proteins after a longer exposure to ionophore in cells loaded with BAPTA and maintained in the absence of extracellular calcium, and compared this with the corresponding level in BAPTA-free control cells. The introduction of BAPTA caused a significant attenuation of the ionophore-induced increase in TCTP, which declined from 8.4-fold to 2.9-fold over basal levels (Figure 7, compare D and E). In contrast, the presence of BAPTA did not affect the ionophore-mediated increase in BiP or endoplasmin in the same cells. Loading of BAPTA and removal of extracellular Ca^{2+} alone did not significantly alter the yield of TCTP (results not shown). Collectively, these results suggest that the expression of TCTP is regulated at a post-transcriptional step in response to the concentration of cytosolic Ca^{2+}.

DISCUSSION

The calcium ionophore A23187 and thapsigargin, a specific inhibitor of the ER membrane Ca^{2+}-ATPase, have been widely used in the study of intracellular events that follow the depletion



	2	2	2	10	10
Ep	1.00±0.14	1.00±0.07	1.00±0.13	4.36±0.52	4.49±0.61
BiP	1.00±0.09	1.00±0.11	1.00±0.11	10.34±1.78	10.56±1.82
TCTP	1.00±0.12	2.40±0.22	1.00±0.08	8.40±1.73	2.93±0.31

Figure 7 Regulation of TCTP synthesis by cytosolic Ca^{2+}

Cos-7 cells were untreated or exposed to ionophore for a period of either 2 h or 10 h in the presence or absence of BAPTA/EGTA as indicated. In each case the cells were labelled with ^{35}S for 2 h immediately before harvesting and aliquots of ^{35}S-labelled protein, normalized for total radioactivity, were resolved by 2-DE. The positions of BiP, endoplasmin (Ep) and TCTP are denoted by arrows. The increase in abundance of each protein relative to the corresponding level in control cells is given below. Mean ± S.E.M. values are from three independent experiments.

of stored Ca^{2+} from the ER. In this study, we have also used expression of a viral glycoprotein to investigate how the programme of gene expression is altered in mammalian cells following the loss of Ca^{2+} homoeostasis after store depletion.

Expression of NSP4 altered the level of synthesis of a group of cellular polypeptides in a manner similar to both A23187 and thapsigargin. The majority of these proteins are members of the class of ER-localized molecular chaperones that are induced in response to a wide variety of perturbants that cause ER stress. In contrast to this group, TCTP was only induced by agents that altered Ca^{2+} homoeostasis (including NSP4 expression), and not by other inducers of the UPR (Figure 4). Therefore we conclude that the signalling pathway(s) utilized to up-regulate TCTP transcription in response to events that occur within the ER is distinct from the UPR and is selectively activated by Ca^{2+} depletion. TCTP may thus prove a useful marker in the molecular dissection of ER–nuclear signalling pathways.

TCTP was initially identified as a tumour-related protein in mouse ascetic tumour and mouse erythroleukaemic cells [33,34]. Subsequently it has been found in a variety of tumour and normal human cells and homologues have been recognized in a number of other species, including nematodes, amphibians, plants and yeast [30]. Despite its ubiquity and remarkable degree of conservation, a unifying physiological role for TCTP has remained elusive. Expression of TCTP, while not exclusively restricted to tumour cells, is growth-related, and several studies demonstrate the induction of TCTP after mitogenic stimulation of mammalian cells [35,36].

As suggested by its name, synthesis of TCTP is recognized to be under translational control [31,32]. The mechanistic details of this process are unclear, but it is known to involve both 5′ and 3′ untranslated regions of the mRNA. A recent report by Sturzenbaum et al. [37] demonstrated that expression of TCTP is also regulated at the level of transcription in the earthworm *Lumbrucus rubellus* following exposure to heavy metals. Potential binding sites for several transcription factors have been identified within the TCTP gene promoter [38], but no data are available on which, if any, of these might play a role in the regulation of TCTP expression *in vivo*. Our experiments now establish TCTP as a protein whose expression is regulated by Ca^{2+} both at the level of transcription and at a post-transcriptional step.

The slow kinetics of mRNA accumulation argues against transactivation resulting from a transient instantaneous release of Ca^{2+} ions from trapped stores (Figure 6). Furthermore, buffering of cytoplasmic Ca^{2+} did not significantly attenuate the increase in TCTP mRNA abundance in response to ionophore. These results demonstrate that depletion of stored Ca^{2+}, but not an increase in $[Ca^{2+}]_i$, results in an increase in the steady-state level of TCTP mRNA. In contrast, elevation of $[Ca^{2+}]_i$ increases TCTP synthesis at a post-transcriptional step. This conclusion is

supported by two experimental findings. First, increased synthesis of the protein was detected in advance of an increase in the steady-state abundance of mRNA after treatment with A23187. Secondly, introduction of the Ca^{2+} buffer BAPTA into the cytosol, before A23187 stimulation, significantly attenuates the ionophore-mediated increase in TCTP synthesis. A trivial explanation for the increase in TCTP abundance following elevation of cytosolic Ca^{2+} could be provided by the possibility that the stability of TCTP is negatively regulated by Ca^{2+}. Pulse–chase experiments exclude this possibility and reveal that the stability of TCTP does not vary (within the standard period of labelling used, 2 h) in response to agents that modulate the level of cytosolic Ca^{2+} (results not shown).

The physiological significance of TCTP induction in response to fluctuations in both ER and cytosolic Ca^{2+} levels is unclear. Given its cytoplasmic location, TCTP is unlikely to be part of a protective response involved in the restoration of normal function to the ER during Ca^{2+} stress. Perturbation of ER can lead to the induction of genes that encode proteins located outside this organelle. For example, DOC6 (downstream of CHOP), which is activated by a variety of ER perturbants, may play an active role in the collapse of the actin cytoskeleton during apoptosis [14]. Since sustained depletion of Ca^{2+} can activate apoptosis in many cell types, TCTP induction could reflect a role for the protein in this process. This suggestion is supported by the recent observation that transcription of the TCTP gene is up-regulated in C6.9 rat glioma cells undergoing apoptosis in response to 1,25-dihydroxyvitamin D_3 [39]. Alternatively, and in view of its Ca^{2+}-binding activity, it is tempting to speculate that the role of TCTP during Ca^{2+} stress might be to sequester increased cytosolic Ca^{2+} and thus delay or prevent the induction of cell death. Further studies are required to define precisely the role of this protein during Ca^{2+} stress and establish its link to the pathogenic state.

Finally, to our knowledge, TCTP represents the first protein whose expression can be regulated by the concentration of Ca^{2+} in different compartments of the cell at both transcriptional and post-transcriptional levels. This novel regulatory feature might represent a mechanism for fine-tuning the expression of the protein in response to physiological requirements. Such a scenario bears similarity to the regulation of iron homoeostasis in mammalian cells via the post-transcriptional control of expression of ferritin and transferrin receptor [40]. In this regard, the identification of a motif within the TCTP mRNA molecule that might confer on Ca^{2+} the ability to regulate its translation represents an immediate objective towards illuminating the regulatory mechanisms that underlie control of TCTP expression *in vivo*.

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